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Journal of the Fisheries Research Board of Canada

Periodic and Resident Components in Communities of Atlantic Fishes

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From trawling at least monthly on a station in Passamaquoddy Bay, N.B., four groups of species were distinguished. One group of species was present on station during winter only, another group in summer only, and a third group was present throughout the year. A fourth group of species was present only occasionally. This type of temporal component structure was found in previously published data for stations with similar habitat but at different latitudes and different temperature regimes. With increased annual temperature fluctuation by locality, there were proportionately more species in the periodic components and fewer in the regular component. Comparable tropical communities had very small periodic components.

Received December 3, 1970

This study examines seasonal changes in relative abundance of fishes caught by otter trawl over a small tract in a deep area of Passamaquoddy Bay, N.B. No similar work has been previously published for the Gulf of Maine and Scotian Shelf region of eastern North America. Patterns of occurrence in Passamaquoddy Bay are compared here with results of previously published studies based farther to the south.

The work is part of a project to examine associations among north temperate marine fishes. Information on seasonal changes in relative abundance of fishes within a locality is necessary background material for studies of functional aspects of associations, such as trophic and spatial relations, and as base-line information in marine pollution studies. One wonders about the impact of large numbers of fishes moving seasonally on trophic associations among resident fishes within a tract; whether there is in fact a set of species present in all seasons.

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Materials and Methods

Fish Collection

Fish were collected by otter trawl on a station 2 miles (3.2 km) long and a mile (1.6 km) wide in Passamaquoddy Bay. Several half-mile tows were made within a day, at least monthly, from March 1965 to June 1966. Sampling dates and number of tows are as follows: March 10, 1965 (6), March 18 (8), April 1 (4), April 21 (2), April 28 (8), May 26 (6), June 22 (8), June 30 (5), August

3 (5) September 7 (2), September 28 (4), October 25 (4), November 17 (5), December 14 (4), January 17, 1966 (4), February 7 (5), March 21 (5), April 18 (5), May 17 (5), May 31 (5), June 27 (5). On each trip, all tows were made within one of five substations within the sampling station. The substations are represented by a series of overlapping rectangles in Fig. 1. Dimensions of the substations were 1 mile (1.6 km) by 0.5 mile. The sequence in which substations were sampled was determined by selecting random numbers — a procedure followed so that the trawl would not continually disturb the same bottom, and so that samples from locations within the station would not be linked with date. Radar buoys were used to outline the sampling area and determine distance towed and towing velocity in relation to ground (2.1–2.5 knots, or 3.9–4.7 km/hr).

The ¾–35 Yankee trawl was modified by lining the belly and lengthening piece with 1-inch (2.5-cm) and the codend with ¼-inch (0.6-cm) knotless nylon mesh (stretch measurement). The bosom section of the roller wire was weighted with a 7.5-ft (229-cm) length of chain with 2-inch (5-cm) links. Steel floats (7 inch (18 cm) diam) were spaced at 24-inch (61-cm) intervals on the headrope. The groundrope of the trawl was 40 ft (12.2 m) long. The trawl was towed by a 45-ft (14 m) 110 hp side-trawler.

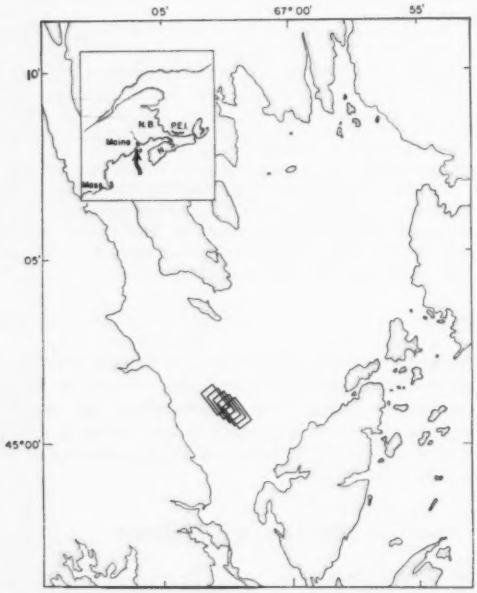

FIG. 1. Sampling station in Passamaquoddy Bay with insert map of the Canadian Atlantic maritime region and northeastern United States. Arrow indicates position of Passamaquoddy Bay. The series of five overlapping rectangular areas with 0.5 × 1.0 mile dimensions are substations.

PHYSICAL ENVIRONMENT

A survey with a bottom grab and echo sounder along a grid with ¼-mile (0.4-km) quadrants indicated that the sampled area had a sloping, brown mud bottom 20–30 fath in depth (38–55 m). Grab samples were taken at grid intersections and the echo sounder was operated along the grid lines.

On each occupation of the station a water sample was collected just over bottom with a Nansen bottle, while temperature was taken with a reversing thermometer attached to it. Specific density of the water was determined with a hydrometer. Density was converted to salinity with a nomograph.

FISH SAMPLING

Usually all fishes caught were measured for length (total length or fork length where the latter was applicable) to the nearest centimeter. Infrequently, especially large numbers of a species were caught. The whole catch was then counted and a random sample of at least 100 fish was measured. For most species, stomach contents and gonad condition were examined for 20 individuals per 10-cm length interval. Trophic relations will be reported in another paper.

Authorities used in identification of fishes were: Bigelow and Schroeder (1953), Leim and Scott (1966), and Templeman (1965). Species nomenclature is after Leim and Scott (1966).

Results and Discussion

TEMPERATURE AND SALINITY

Both temperature and salinity progressed through a seasonal cycle (Fig. 2). The lowest temperature (1.2 C) occurred in February. During January, February, and March, the temperature remained between 1.2 and 1.6 C. Temperature rose in the spring and summer to a peak of 10.1 C at the end of September. From August 3 to October 25 the temperature remained between 8.9 and 10.1 C.

The lowest salinities, 29.5 and 29.6‰, occurred during March, April, and May. The highest salinity, 32.3‰, occurred on September 7.

RELATIVE ABUNDANCE OF FISHES

Some species were present only during the warm months (Fig. 3) and will be referred to as "summer periodics." All summer periodics were present when the temperature peaked in September. Silver hake (*Merluccius bilinearis*), white hake (*Urophycis tenuis*), and fourbeard rockling (*Enchelyopus cimbrius*) moved into the area at the end of June. Silver hake were gone by November, and fourbeard rockling by December. White hake rapidly decreased in number from November to December, were scarce in December, January, and February, and

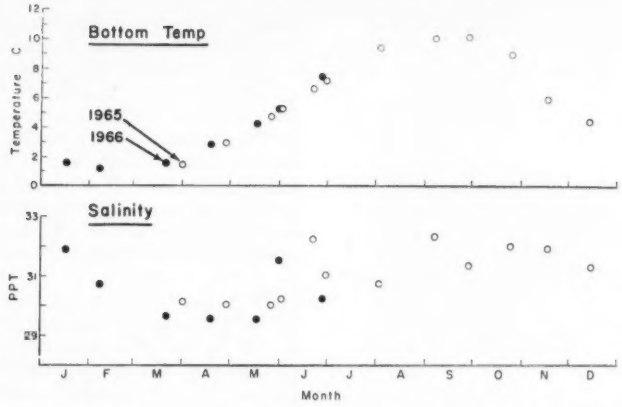

FIG. 2. Bottom temperature and salinity on station for each sampling date from March 1965 to June 1966.

were not present after February. The other summer periodic was the alewife (*Alosa pseudoharengus*). A few stragglers were caught in April and June 1965, but most entered the area in September and remained through October. They diminished in number during November and were absent thereafter.

"Winter periodics" were those species that occurred during the annual temperature minimum, and on several consecutive sampling dates during the cold period, but were absent during the warm period. Pollock (*Pollachius virens*) and winter skate (*Raja ocellata*) entered the area in December and left after March (Fig. 4). Little skate (*Raja erinacea*), the most abundant winter periodic, entered the study area in November and left the following April. A few stragglers of this species were caught in May and June. Data on the fourth winter periodic, capelin (*Mallotus villosus*), were omitted from Fig. 4 to retain clarity in the figure. Capelin were not caught in March 1965, but were caught at rates of 5.5, 10.0, 0.2 fish per half-mile tow on April 21, 28, and May 26, respectively. Capelin did not show up again in catches until November 17, 1965 (0.6 per half-mile tow), December 14 (0.5 per half-mile tow), February 7 (1.2 per half-mile tow), and March 21 (0.4 per half-mile tow). They were not caught in April, May, or June 1966 samples. Capelin do not usually occur in Passamaquoddy Bay (Tibbo and Humphreys 1966).

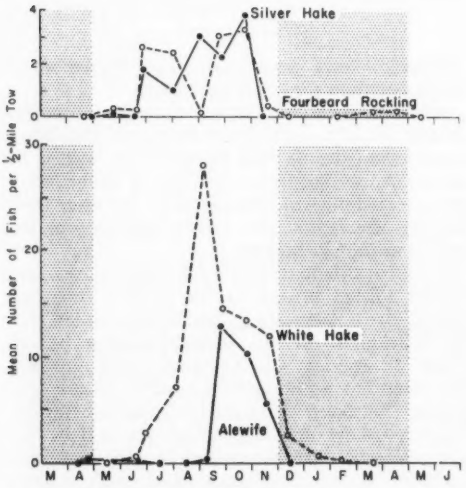

FIG. 3. Relative abundance of fishes of the summer periodic community component.

"Regulars" were species that were present on every, or nearly every, sampling date. Continued presence was used as the classifying criterion rather than abundance fluctuations. Species that showed seasonal fluctuation in their abundance but nevertheless remained common throughout the year, and fed in the area throughout the year, were grouped together with species of constant abundance because all played a continuous part in community trophic dynamics. The term "seasonals" has been used in other publications to describe species with abundance fluctuations as well as species that are entirely absent during a part of the year. The two groups of seasonals should be separated when ultimate interest is in community energetics — the manner in which the fishes divide and utilize a food resource.

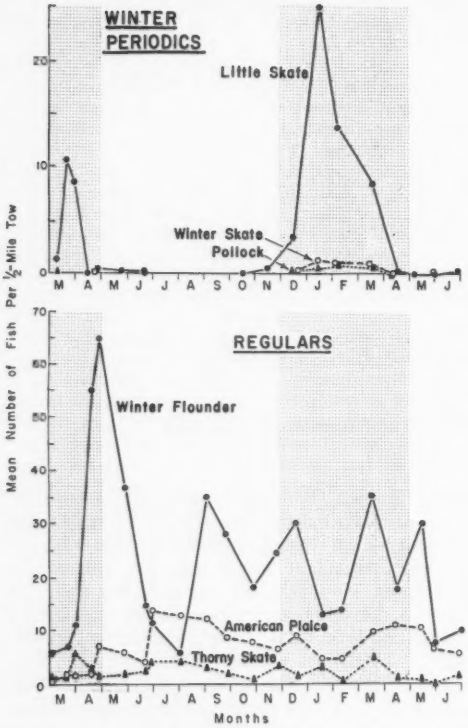

FIG. 4. Relative abundance of fishes of the winter periodic and regular community components.

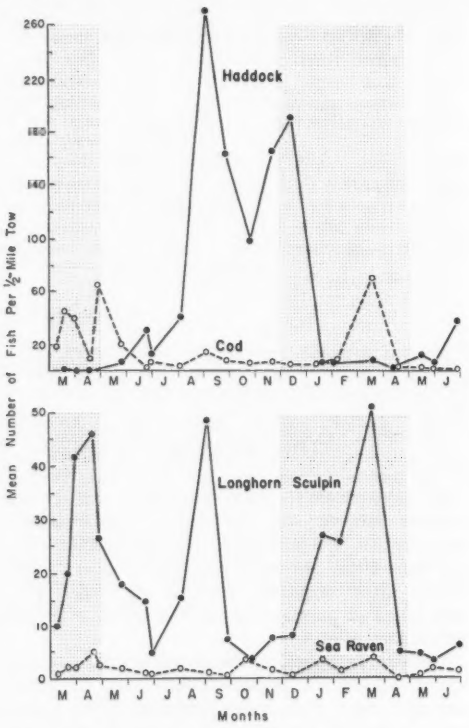

FIG. 5. Relative abundance of fishes of the regular community component.

American plaice (*Hippoglossoides platessoides*), thorny skate (*Raja radiata*), Atlantic sea raven (*Hemitripterus americanus*), alligatorfish (*Aspidophoroides monopterygius*), and redfish (*Sebastes marinus*) all show remarkably even catch rates through the year (Fig. 4–6). However, few of the regulars were more abundant during the cold months. Longhorn sculpin (*Myoxocephalus octodecemspinosus*) were caught at higher rates during January, February, and March. There was, however, an isolated high catch rate at the beginning of September. Atlantic Cod (*Gadus morhua*) were more plentiful in March and April, winter flounder (*Pseudopleuronectes americanus*) in April and May, snakeblenny (*Lumpenus lumpretaeformis*) in April, May, and June. Other regulars showed higher catch rates during warm months. Ocean pout (*Macrozoarces americanus*) were more abundant from April through September, rainbow smelt (*Osmerus mordax*) from the end of June through September. There were much higher catch rates for haddock (*Melanogrammus aeglefinus*) from September to December than for other months.

Atlantic herring (*Clupea harengus harengus*) showed variable catch rates but were regulars in occurrence (Fig. 6). The largest catch was at the end of April 1965. The variation is likely due to the inaccessibility of this species to the otter trawl. Herring often form tight schools that may or may not be at the sea bottom.

A fourth component of the community consisted of those fishes present in the samples irregularly and in low numbers (with one exception, less than 14 individuals for the entire sampling period). These 18 species will be termed "occasionals," and occurred as follows: barndoor skate (*Raja laevis*), butterfish (*Peprilus triacanthus*), grubby (*Myoxocephalus aenaeus*), moustache sculpin (*Triglops murrayi*), shorthorn sculpin (*Myoxocephalus scorpius*), goosefish (*Lophius americanus*), Atlantic tomcod (*Microgadus tomcod*), wrymouth (*Cryptacanthodes maculatus*), one or two specimens;

Atlantic hagfish (*Myxine glutinosa*), arctic hookear sculpin (*Artediellus uncinatus*), lumpfish (*Cyclopterus lumpus*), rock gunnel (*Pholis gunnellus*), radiated shanny (*Ulvaria subbifurcata*), Atlantic halibut (*Hippoglossus hippoglossus*), yellowtail flounder (*Limanda ferruginea*), three to eight specimens; smooth skate (*Raja senta*) 10 specimens; witch flounder (*Glyptocephalus cynoglossus*), 13 specimens; daubed shanny (*Lumpenus maculatus*), 45 specimens. These fish might have been rare but always present, or present only occasionally in the area, or inaccessible to the trawl.

Through the 16-month sampling period there were 13 regulars, 4 summer periodics, 4 winter periodics, and 18 occasionals. Changes by date in the numbers of fish species of the periodic (summer and winter together), regular, and occasional community components are shown in Fig. 7. On any one date there were generally 10–13 regulars, and 4 or 5 periodics of either the winter or summer group. The number of occasionals fluctuated from zero to seven. A paucity of species in April and May occurred in both the occasional and periodic components. Figures 3 and 4 show that by April winter periodics had left the study area and the summer periodics had not yet arrived. This was the time of year when salinity was lowest and temperature was beginning to rise. In the autumn there was no time when both winter and summer periodics were absent. On the contrary, the transition was made with a partial overlap between the two components.

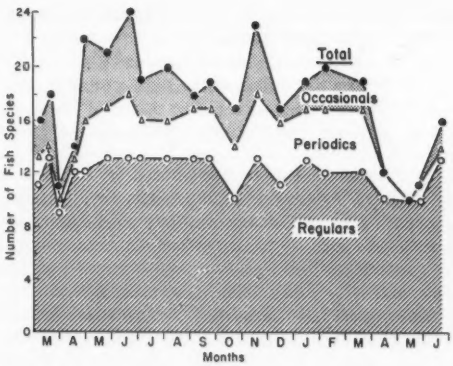

FIG. 7. Number of fish species collected on each sampling date. The height of the diagonally shaded area represents the number of species of the regular community component; the height of the middle unshaded area, the number of periodic species of both winter or summer components; the width of the stippled area, the number of species of the occasional component; the top line of points, the total number of species present on each sampling date.

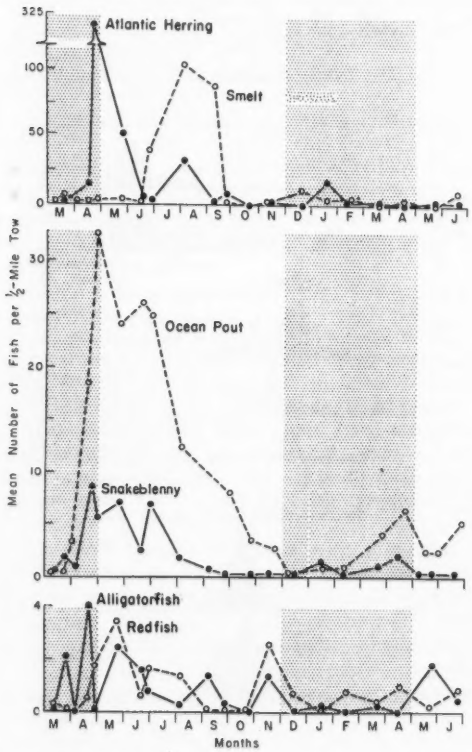

FIG. 6. Relative abundance of fishes of the regular community component.

LENGTH-FREQUENCY DISTRIBUTIONS

Consideration of length-frequency distributions of abundant fishes is included in this paper because it is as important to know which sizes were present as which species were present. However, it is well known that escape size of fishes from trawls depends upon mesh size and material, and fish shape (McCracken 1963). Fifty percent of the 8-cm long haddock and cod will escape through 1-inch (2.5-cm) mesh, as will 50% of the 5-cm redfish and plaice (Templeman 1963). Since the codend for the present study was lined with ¼-inch (0.6-cm) mesh, fish of only a few centimeters length should be retained there. However, since the 1-inch liner in the other sections of the trawl would allow these small fishes to escape, there should be disproportionately fewer fishes of sizes less than 5–8 cm. Eel-like fishes would escape more easily than would haddock and redfish,

however, and 50% retention length would be greater than 8 cm for these slender forms.

White hake showed a striking length-frequency change during the course of the summer and autumn (Fig. 8). Larger fish with a size range of 26–43 cm moved into the area in early August. By September 7, a few small fish (15–25 cm) had moved into the area. The small fish had increased in number by the end of September and became the dominant group of hake in October and November. Towards the end of October, the large fish began to diminish in abundance. In December, there were no larger hake and only a few small hake were left.

Movements of large fish independent of the movements of the small fish of a species were not evident in species other than white hake. Except for growth, the date-by-date length-frequency plots of all other species varied little. Length-frequency histograms of single samples of these species are given in Fig. 9. The dates chosen for display are those on which the largest samples were measured.

The length-frequency distributions of winter skate, thorny skate, Atlantic sea raven, and pollock were not included in Fig. 9, because sizes of individuals were rather evenly distributed over the length ranges recorded. Length ranges for the entire sampling period are as follows: winter skate 39–83 cm, thorny skate 11–73 cm, Atlantic sea raven 4–49 cm, and pollock 15–27 cm.

Length-frequency distributions of capelin were similar to those shown by Tibbo and Humphreys (1966) for Bay of Fundy locations in 1965. The mean length and length range for unsexed Passamaquoddy capelin caught during the present study were 14.1 cm and 13–19 cm.

Winter skate, thorny skate, little skate, snakeblenny, ocean pout, fourbeard rockling, winter

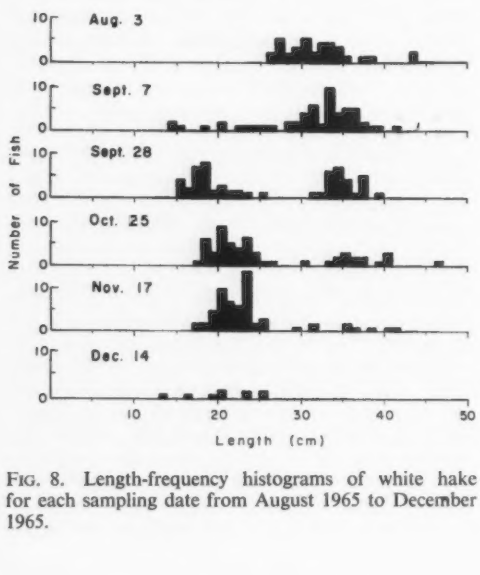

FIG. 8. Length-frequency histograms of white hake for each sampling date from August 1965 to December 1965.

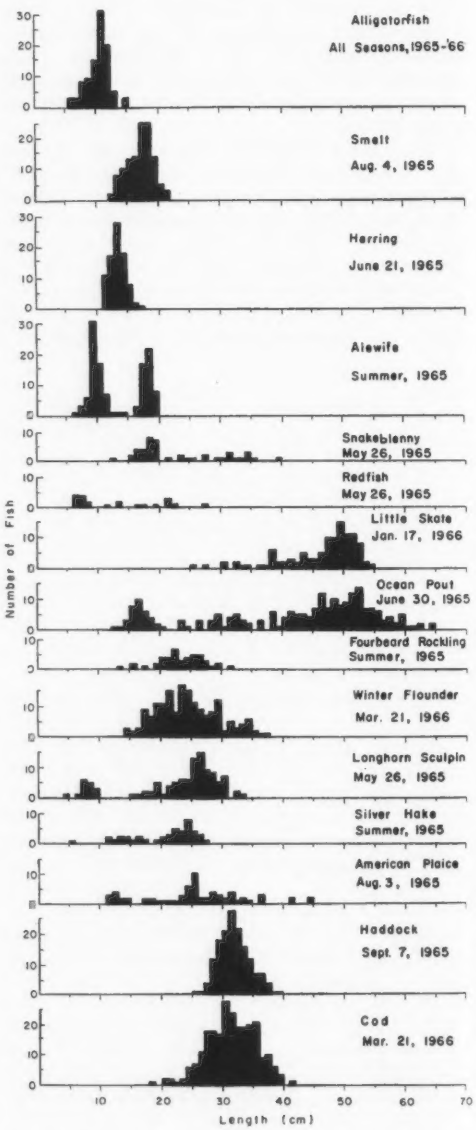

FIG. 9. Length-frequency histograms of fishes of the regular and periodic community components.

flounder, longhorn sculpin, Atlantic sea raven, alligatorfish, and rainbow smelt were large enough to be present as mature fish (Bigelow and Schroeder 1953). On the other hand, white hake, haddock, Atlantic cod, American plaice, silver hake, Atlantic herring, alewife, pollock, and redfish were mostly immatures.

Abundance Changes and Spawning

Except for three species, winter flounder, fourbeard rockling, and capelin, there seems to be little relation between spawning period and periods of seasonal abundance. An attempt was made to classify gonads of species represented by mature individuals into ripening and spawning stages by blood vessel size and ovary color, as defined by Powles (1958) for Atlantic cod, but the attempt was successful for winter flounder only. Winter flounder were particularly abundant at the end of April 1965, and at that time they were in a spawning and just-spawned condition. It seems doubtful that these fish were depositing eggs on the mud bottom of the sampling station. It is more likely that they were passing through to deposit their adhesive eggs in nearby, shallow rocky areas (about 1.5 miles or 2.4 km distant).

Fourbeard rockling were present on station only during the period in which they spawn. They were summer seasonals and their eggs have been found in plankton tows in Passamaquoddy Bay in summer (Battle 1930). Since they have buoyant eggs they possibly spawn over the mud bottom of the trawling station.

Ocean pout are reported to spawn in September and October (Leim and Scott 1966). However, this species showed maximum abundance in early summer (Fig. 6). Longhorn sculpin (winter spawners) were generally more abundant in winter, except for somewhat isolated high catches on September 7 (Fig. 5). However, these fishes have adhesive, nonfloating eggs that are most likely deposited on hard bottom rather than the soft mud of the sampling station, and so were probably not spawning on station.

The only other adult fishes with pronounced seasonal abundance changes that were examined for maturity condition were winter skate, little skate, and thorny skate. None of these fish that were caught contained egg cases or running milt.

Although none of the capelin reported here as winter periodics were examined for maturity condition, Tibbo and Humphreys (1966) examined fish caught nearby in April 1965, and found that they were in a prespawning condition. This condition, combined with the predominance of males, led these authors to suggest that the capelin were in a prespawning migration. Templeman (1947) found that there was a partial separation of sexes in prespawning capelin schools.

Comparisons of Marine Fish Assemblages by Latitude

There were three previously published studies with data on changes in relative abundance of fish throughout the year at single, deepwater, inshore stations on the east coast of North America. Merriman and Warfel (1948) gave complete relative abundance data for 3 years for all 43 species caught by a 50-ft (15-m) dragger working a small fishing ground in Block Island Sound (Watch Hill to Point Judith inshore of Block Island). Data reviewed in the present paper are those from December 1943 to December 1944. Their samples came from a trawl with an 80-ft (24-m) groundrope and with either 2.5- or 4.5-inch mesh (6–11 cm) The salinity of the area was fairly constant at about 32‰. Depth ranged from 18 to 46 m.

Richards (1963) gave complete relative abundance data for all 24 species of fish caught throughout a 1-year period by a small dragger in Long Island Sound. Twenty-five tows of 30 min duration were spread fairly evenly over a year's time (1956–57). Data from Richards' station 3A will be examined here because of the mud bottom (similar to the Passamaquoddy station). Also, of Richards' two stations, 3A had the greater depth (17 m). Her data are not entirely comparable to my Passamaquoddy data, however, since most large fish probably could avoid her small trawl (20 ft, or 6 m, groundrope). Richards felt that "A thorough study of the adult population would have required a larger boat capable of accommodating commercial-sized gear." The trawl was of 1-inch mesh and a ¼-inch liner in the codend. Salinity near Richards' station 1 ranged from 24 to 28‰, reaching the minimum in April and May (Riley 1955).

Massman (1962) presented trawling data for a series of stations in the mouth of the Chesapeake Bay and York River estuary that were sampled for 44 months. One 15-min tow was made at each station each month. Samples were taken with an otter trawl having a 30-ft (9-m) groundrope and with 1-inch mesh in the wings and body, ¾-inch mesh in the codend. Massman's stations 1–4 (in the bay mouth) are comparable to the Passamaquoddy Bay station. The combined data for stations 1–4 for the period December 1957 to December 1958 are considered here. Salinity was generally 28‰ and water depth was 11–15 m. Sixty-two fish species were collected at the Chesapeake Bay mouth stations.

Annual temperature changes in the Block Island Sound, Long Island Sound, and Chesapeake Bay stations are shown in Fig. 10.

I have arranged the data from Merriman and Warfel, Richards, and Massman into temporal components in Tables 1, 2, and 3, respectively. Regular, periodic (winter and summer), and occasional groups were evident. There were no species from these stations avoiding both the summer maximum and winter minimum and thus present in the spring and fall only. As with Passamaquoddy Bay, the spring and fall fishes were transitional between summer and winter assemblages in Block Island Sound, Long Island Sound, and Chesapeake Bay mouth. It should be noted in passing that assemblages of fishes that can be seined on beaches do have groups of species present in spring and fall only (Tagatz and Dudley 1961; Warfel and Merriman 1944).

The degree of similarity among the species lists of the regular and periodic components of the four areas can be compared by calculating the percentage of species in common, or percentage overlap, between adjacent stations. Thirty percent of the species, comprising the regular and periodic components of Passamaquoddy Bay and Block Island Sound, occur in both areas. The regular and periodic components for Block Island Sound and Long Island Sound stations overlap by 25%, and the lists for the Long Island Sound and Chesapeake Bay stations overlap by 15%.

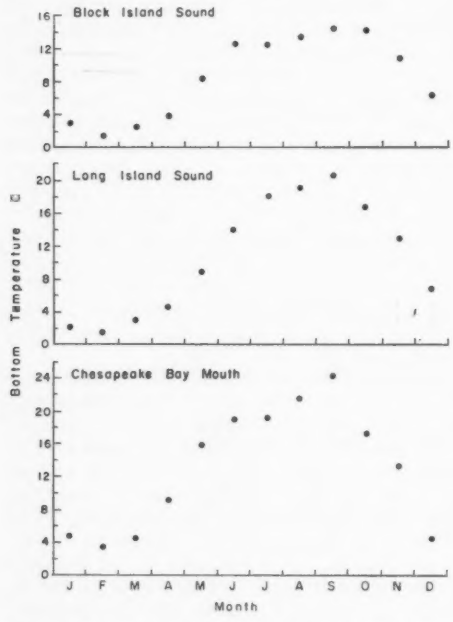

FIG. 10. Bottom temperatures on stations Block Island Sound, Long Island Sound, and Chesapeake Bay plotted from data by Merriman and Warfel (1948), Richards (1948), and Massman (1962), respectively.

The Passamaquoddy assemblage has progressively less in common with the other assemblages as one proceeds south. There is a 30% overlap with Block Island Sound, a 14% overlap with Long Island Sound, and a 3% overlap (one species, *Alosa pseudoharengus*) with the Chesapeake Bay mouth.

The proportion of the species among regular, periodic, and occasional components changes with latitude and annual temperature range. As one proceeds from Passamaquoddy Bay to Chesapeake Bay, the annual temperature fluctuation increases, the proportion of regulars drops, the proportion of periodics increases, and the proportion of occasionals increases (Fig. 11). The temperature range of Passamaquoddy Bay was 9 C; of Block Island Sound, 13 C; of Long Island Sound, 19 C; of Chesapeake Bay mouth, 20 C. The chief difference between Chesapeake Bay mouth and Long Island Sound was the duration of temperatures 19 C and higher. The high temperatures prevailed for 1 month in Long Island Sound and 4 months in Chesapeake Bay mouth (Fig. 10). Instability in the temperature regime apparently brings about instability in the fish species assemblages, in that the regular component becomes progressively smaller as annual temperature fluctuation increases.

TROPICAL ASSEMBLAGES OF DEMERSAL FISHES

If presence of temporal components is largely due to annual temperature fluctuation, then assemblages of tropical groundfish living under fairly constant temperature conditions should not show these components. In making such comparisons between tropical and temperate faunas one must make certain that the tropical region in the comparison does actually have a constant temperature regime. Longhurst (1964) points out that some areas in tropical west Africa are subject to seasonal variations in upwelling that bring about large temperature changes. Four trawling surveys in tropical Atlantic waters are available for comparison, although the complete relative abundance data by season and species have not been published. A major trawling survey was conducted off the coast of British Guiana from 1957 to 1959 (1070 drags) (Mitchell and McConnell 1959; Lowe 1962). Tows were made at depths from 9 to 185 m with effort concentrated between 9 and 75 m. Sea surface temperatures in the area generally

TABLE 1. Regulars and periodics from Block Island Sound. Numbers of fish per 1-hr tow. Data from Merriman and Warfel (1948).

	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Regulars													
Raja ocellata	3	6	4	10	7	·	15	6	14	20	17	10	20
Raja erinacea	13	16	6	19	61	21	111	40	356	219	65	19	90
Pseudopleuronectes americanus	62	61	69	75	500	235	1245	2814	1762	861	287	537	30
Scophthalmus aquosus	64	102	40	42	9	1	3	12	102	95	56	68	98
Myoxocephalus octodecemspinosus	530	95	15	159	594	100	39	14	8	9	210	720	116
Hemitripterus americanus	1	1	·	1	·	1	4	8	4	·	7	7	6
Winter seasonals													
Gadus morhua	40	2	5	1	4	·	·	·	·	·	2	7	60
Limanda ferruginea	5	1	3	1	5	·	·	·	2	·	·	7	6
Macrozoarces americanus	1	·	1	2	21	1	·	·	·	·	·	·	6
Summer seasonals													
Raja laevis	·	·	·	·	1	·	9	4	50	3	·	·	2
Squalus acanthias	·	·	·	·	·	·	·	2	14	2	·	·	·
Merluccius bilinearis	22	·	·	·	·	13	36	306	344	464	477	233	438
Urophycis chuss	·	·	·	·	9	2	1	130	32	75	2	·	6
Urophycis tenuis	·	·	·	·	·	·	·	42	6	12	·	·	10
Stenotomus chrysops	·	·	·	·	·	1	1	·	·	27	·	·	·
Tautogolabrus adspersus	·	·	·	·	1	11	3	8	24	9	·	·	·
Paralichthys oblongus	·	·	·	·	·	2	17	60	138	134	22	·	·
Paralichthys dentatus	·	·	·	·	·	·	3	·	8	9	3	·	·

TABLE 2. Regulars and periodics from Long Island Sound. Numbers of fish per ½-hr tow. Data from Richards (1963).

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Regulars												
Merluccius bilinearis	3	33	6	4	5	38	28	17	3	57	2	29
Urophycis chuss	·	·	1	1	2	5	5	6	·	43	11	7
Scophthalmus aquosus	18	3	3	8	3	9	7	13	1	23	4	3
Pseudopleuronectes americanus	30	11	8	34	83	20	36	58	4	287	41	17
Winter seasonals												
Clupea harengus	1	9	12	2	·	·	·	·	·	·	·	·
Menidia menidia	1	1	·	1	·	·	·	·	·	·	·	·
Summer seasonals												
Enchelyopus cimbrius	·	1	·	·	·	·	4	4	·	2	1	3
Urophycis regius	1	·	·	6	2	4	5	·	1	·	·	·
Cynoscion regalis	·	·	·	·	·	·	·	·	20	15	·	·
Tautogolabrus adspersus	·	·	·	7	1	2	10	16	·	3	3	·
Prionotus carolinus	·	·	·	·	2	·	·	·	·	16	3	1
Paralichthys oblongus	·	·	·	·	·	2	·	·	1	14	1	·

TABLE. 3. Regulars and periodics from Chesapeake Bay mouth. Numbers of fish per hour tow. Data from Massman (1962).

	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Regulars													
Anchoa mitchilli	8918	42	62	1	48	1002	192	5	1	59	625	1643	72
Paralichthys dentatus	4	·	1	·	1	2	1	4	·	·	·	3	1
Winter seasonals													
Brevoortia tyrannus	13	5	2	·	·	157	·	·	1	·	1	4	64
Alosa pseudoharengus	·	1	1	2	1	·	·	·	·	·	·	2	745
Alosa aestivalis	1	800	1	2	·	·	·	·	·	·	·	·	780
Urophycis regius	11	·	1	·	7	104	19	·	·	·	·	·	·
Syngnathus fuscus	10	·	2	·	·	·	·	·	·	·	·	·	1
Summer seasonals													
Anchoa hepsetus	·	·	·	·	·	·	3	·	25	45	5	·	·
Bairdiella chrysura	10	·	·	·	·	27	6	·	·	88	43	45	·
Cynoscion regalis	13	·	·	·	·	7	33	·	521	24	90	8	·
Leiostomus xanthurus	10	·	·	·	1	1	1	1	87	·	9	29	·
Micropogon undulatus	31	·	·	·	·	·	·	·	·	·	·	1	2
Stenotomus chrysops	·	·	·	·	·	4	·	·	4	5	3	·	·
Prionotus carolinus	·	·	·	·	·	4	·	·	1	·	·	1	·
Poronotus triacanthus	·	·	·	·	·	31	1	·	47	723	26	4	·
Sphaeroides maculatus	·	·	·	·	·	·	2	·	·	·	·	·	·
Trinectes maculatus	·	1	·	·	·	·	5	2	1	·	1	6	·
Symphura plagiusa	5	·	·	·	·	·	·	·	·	·	1	1	·
Etropus microstomus	16	·	·	·	·	1	1	3	1	·	1	4	2

range from 26.5 to 27.5 C annually (Mazeika 1968). The dominant species in the latter depth range were sciaenids. None of the fishes occurred

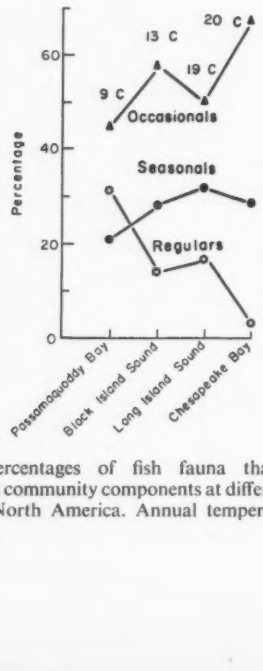

FIG. 11. Percentages of fish fauna that comprise the temporal community components at different latitudes in eastern North America. Annual temperature ranges are at top.

in the temperate communities reviewed in the present paper.

McConnell reported that date-by-date variation in relative abundance of species was irregular and was not strongly related to time of year. Some movement towards the rivers was evidenced from June to August during the heavy rains when river flow was greatly enhanced.

Three species made up 73% of the total weight of the catch over the 2-year sampling period. Monthly catch records of only these species were published. *Macrodon ancylodon* and *Cynoscion virescens* catches showed no correlation with season. *Micropogon furnieri* was somewhat less abundant in December, January, and February than in other months.

Rathjen et al. (1969) assessed seasonal variation in catches of marketable fish by two research trawlers working a large area off northeastern South America. These vessels completed over 3000 otter trawl drags from Trinidad to French Guiana at depths of 9–185 m from 1962 to 1965 and from 1967 to 1968. The fauna was largely the same as covered in Mitchell and McConnell's work. It was associated with mud bottom and depths of less than 40 m. The authors reported that catch composition of principal species changed little with season, though catch rates varied. Off Surinam,

highest catches were in July corresponding to maximum flow of the Surinam River. *Micropogon furnieri* comprised an average of 4% of the total catch (by weight) in March, April, May; 13% in June, July, August; 8% in September, October, November; and 3% in December, January, and February. *Cynoscion virescens* comprised slightly over 50% of the catch from September to May and 42% during June, July, and August.

Longhurst (1963) and Fager and Longhurst (1968) published definitive studies of marine fish assemblages in tropical west Africa (approximately 10°N to 10°S of the equator). As in the tropical west Atlantic, the demersal west African fish fauna, in less than 40 m of water, was dominated by sciaenids, but by species not found in the western Atlantic. They were associated with mud bottom and temperatures of 25–27 C.

Longhurst (1963) reported that there were seasonal migrations of at least some species at the northern and southern ends of the west African tropical area related to temperature fluctuation. Fager and Longhurst (1968) found that the mean depth of the sciaenid fauna as a whole was somewhat deeper during the warmer months, and they felt that this was "slight evidence" that at least some species regularly shifted with season. However, Longhurst (1963) gave extensive trawling data for a 3-year period on a station in the Sierra Leone estuary showing that there was no seasonal change in catch composition among principal species, except for *Arius* (= *Tachysurus*), which reached maximum abundance when salinity reached the annual low. Temperature ranged from 26.3 to 28.8 C annually at the mouth of the estuary (Watts 1958).

Durand (1967) gave data from 137 trawl sets made on a transect off Pointe-Noire, Congo, during the period 1963–65. The inshore fish fauna was similar in species composition to the fauna reported on by Longhurst (1963). In contrast to the Sierra Leone estuary, however, water temperatures on the bottom had an annual variation of about 10 degrees C: 18–28 C at 15–30 m depth (Poinsard and Troadec 1967). Of 16 common species, five were rare or absent during the cool months. The situation was similar in this respect to Passamaquoddy Bay, where there was a 9-degree C annual variation in water temperature and four species left the area during the cold months. Off Pointe-Noire there were no species that occupied the inshore area specifically during the cold season as there were in Passamaquoddy Bay.

Some tropical demersal fish assemblages, thus, do not have temporal components superimposed on regular components as do temperate assemblages. The few species in these areas that form an exception seem to move in response to river flooding, which brings less temperature change than salinity change. Since large temporal components do not exist in these species assemblages subjected to a constant temperature regime, the thesis that it is mainly temperature fluctuation that forms temporal components is supported.

Conclusions

In the temperate regions, inshore, deepwater fish communities are made up of regular, summer, and winter periodic components. There are also numerous occasional species. Spring and fall seasons are transitional with regard to fish fauna. Formation of temporal components is largely related to temperature regime, though presence or absence of some species cannot be explained by temperature alone. At latitudes where there is relatively greater annual temperature fluctuation, there are proportionately more species in the temporal components and fewer in the regular component. In this sense, stability of the community is directly related to stability of the temperature regime. Some inshore, tropical, demersal communities have small temporal components or are lacking temporal components, indicating that in the absence of radical temperature fluctuation, these components tend to disappear.

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Effects of Blood Pathway on the Blood-Pressure Drop in Fish Gills, With Special Reference to Tunas[1]

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In tuna gills, blood flows obliquely to the long axis of the secondary lamellae rather than with it as in other teleosts. This pathway greatly shortens the length of the small diameter respiratory vessels; it shortens them by a factor of 4 in a 1.67-kg skipjack tuna (*Euthynnus pelamis*) and of 9 in a 272-kg bluefin tuna (*Thunnus thynnus*). Total blood-pressure drop across the entire gill varies as the square of the length of these vessels, so the drop is reduced by a factor of 16 in the skipjack and of about 80 in the bluefin tuna. Blood-pressure drop also varies as the inverse of the cube of the vessel diameter, and several teleosts we examined showed a compensating increase in diameter as length increased with fish size. Because of the shorter vessel lengths, the diameters seen in tunas are relatively small.

Vessel diameter determines, considerably, the thickness of the secondary lamellae. The specialized pathway of the tunas, in addition to reducing blood pressure drop, allows the secondary lamellae to remain relatively very thin. For a given lamellar spacing, this maximizes the interlamellar water channel thickness and minimizes resistance to water flow. The specialization is a major change in "design strategy" and its advantage appears to lie with the balance between the energy requirements of moving water and blood. Some such change may be essential to the attainment of high levels of activity in fish of large size.

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THE respiratory blood pathway in the secondary lamellae of tuna gills appears to be unique among teleosts. The flow of blood in other teleosts is generally with the long axis of the lamella (here, lamella is used synonymously with secondary lamella), but in tunas a parallel series of vessels carry the blood along the "free" border of the lamella and distribute it to the respiratory channels that run obliquely across the lamella. The respiratory water pathway is not likely to be fully countercurrent to the blood pathway and for large tunas we believe it must be obliquely crosscurrent. Analyses of the gas exchange for both countercurrent and crosscurrent flows have been made and will be reported subsequently.

In the present report, we describe the blood pathway observed in three species of tuna and compare measurements of the blood channel length, with respect to lamella size, with that for several other teleosts. Finally we derive an equation for blood pressure drop as a function of blood channel length and apply this to the tuna data. Implications from the equation are tested with data from cod, trout, and salmon.

The cross-flow system in the secondary lamellae of tunas is not to be confused with the serial multicapillary system postulated for elasmobranchs by Piiper and Schumann (1967), for the similarity does not extend beyond a generalized description of flow directions. Whereas in tuna the water flows across a series of blood channels within a single secondary lamella, Piiper and Schumann suggest that in elasmobranchs it flows across a series of secondary lamellae, within each of which the flow may be countercurrent. (The reality of the multicapillary system in elasmobranchs is not necessarily accepted (Hanson and Johansen 1970).) Piiper and Baumgarten-Schumann (1968) point out that the theoretical maximum efficiency of a serial multicapillary system is lower than that

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of an ideal countercurrent system. The tuna system is a modification of the basic countercurrent plan seen in other teleosts, so we assume the less efficient system has been favored in the interest of the total efficiency of the organism. We believe that a major advantage of this system, to the tuna, is a reduction in cardiac work by minimizing the blood pressure drop across the secondary lamellae. Because of the interaction between the geometry of the blood and water pathways and the total gill surface area, the modification may also be of advantage in preventing the cost of irrigation from becoming excessive with increasing fish size.

Materials

Sources of most of the gills used in this study (skipjack tuna (*Euthynnus pelamis*), yellowfin tuna (*Thunnus albacares*), bluefin tuna (*Thunnus thynnus*), and Atlantic cod (*Gadus morhua*)) have been listed in an earlier report (Muir 1970). Gills from specimens of *Salmo gairdneri* were obtained from Sea Pool Fisheries Limited, and of *Salmo salar* from commercial nets, both in Nova Scotia. All material was fixed in 10% formalin.

For each specimen a filament was selected from near the middle of the first arch and the number of secondary lamellae per millimeter of filament determined near its middle. Several secondary lamellae were dissected out from that location and flooded with Geimsa stain on a glass slide. Measurements were made of the length of lamella, width near the middle, and angle and length of the respiratory blood channels near the middle of the lamella. The lamellae are most uniform in this region and specimen comparison is possible using this technique. (For a skipjack tuna studied in detail (Muir and Hughes 1969), lamellae in this region were about the same size as the average for the entire gill, although smaller than the largest that could be found.)

Results

Each gill arch supports several hundred filaments, which bear the respiratory secondary lamellae. Detailed description of the anatomical arrangement has been given earlier (Muir 1970). For present orientation, the diagram in Fig. 1 represents a cross section through three filaments and shows partial detail of the blood pathway on two secondary lamellae.

Blood Pathway in Secondary Lamellae of Tuna

A secondary lamella from a 62-cm skipjack is shown in Fig. 2A. The lamella was attached to the filament along the right-hand edge. The top one-quarter of the left-hand edge (which is completely free in other teleosts) was fused to adjacent lamellae of the same filament and lamellae of the adjacent filament (Muir and Kendall 1968) (Fig. 2C, D.)

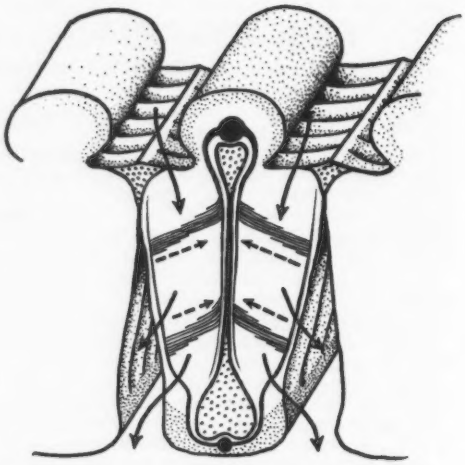

Fig. 1. Diagram of a cross section through three filaments of a tuna gill showing direction of blood flow (broken arrows) for two secondary lamellae and direction of water flow (solid arrows).

The upper left corner was lost in this preparation (Fig. 2A) because of difficulty in separating the fusion. The longitudinal folds seen in the central portion are the result of attempting to flatten the curved lamella. This curvature is considered in our analysis of the water pathway (Brown and Muir 1970, and in preparation).

The general features of the tuna blood pathway have been described earlier (Muir 1970). The afferent lamellar vessel divides into a series of parallel large channels (lower left in Fig. 2A) that are separated by cells resembling pillar cells. These channels, which might be termed "feeder channels," give rise to groups of obliquely running, smaller, respiratory channels. In Fig. 2A, the red blood cells are dark and the pillar cells, separating the blood channels, are evidenced only by the light spaces. Blood is collected along the right-hand side by a single vessel that becomes, or perhaps is, the efferent lamellar vessel.

The appearance of this lamella is fairly typical of 10 skipjack examined. The apparent congestion of blood in the upper portion (see enlargement, Fig. 2B) and abrupt change to a highly organized flow to the right of it were seen in them all. The degree of interchannel cross flow of red blood cells in the remainder of the field and the number of red blood cells in each channel were variable. On greater magnification, channel detail for skipjack tuna is virtually undistinguishable from that shown in Fig. 3 for bluefin tuna. In no tuna speci-

men were areas of the secondary lamella essentially devoid of red blood cells as was found in the cod and trout we examined. With the exception of the congestion noted above, red blood cells were evenly distributed over the entire field. No evidence for nonrespiratory shunts (Steen and Kruysse 1964) was observed in the tuna.

Column 7 in Table 1 gives the angle of the blood channels in relation to the long axis of the lamella. For the three species of tuna, the channels have rotated a consistent 50–60° from the long axis direction of flow. Small lamellae from near the tip of the arch of a skipjack tuna showed an angle of less than 18°, so the rotation may increase with increasing lamella size up to a limit of about 60°. Some degree of rotation was observed in mackerel, marlin, and swordfish (Table 1). No rotation was seen in dolphin, wahoo, or cod (or in the salmonids that are not included in this part of the study). Especially in the large cod a curvature in the channels was observed, associated with the semicircular shape of the free border of the lamella, but the channels essentially ran the length of the lamella in the typical teleost manner.

TABLE 1. Lamellar measurements for several teleosts.

Fork length	Wt	Filament length (*cm*)	No. lamellae/mm	Lamella length (*mm*)	Lamella width (*mm*)	Angle of blood channels	Blood channel length (*mm*)
Skipjack tuna (*Euthynnus pelamis*)							
37.5	965	2.2	32.3	1.52	0.12	58	0.25
37.7	983	2.4	38.2	1.16	0.20	56	0.30
38.5	1056	2.5	34.4	1.06	0.17	50	0.30
41.2	1326	2.3	32.6	1.42	0.20	63	0.34
47.7	2172	3.4	34.7	1.42	0.24	55	0.38
50.0	2546	3.4	32.8	1.56	0.22	60	0.37
51.2	2758	3.5	35.9	1.66	0.18	53	0.32
56.2	3764	3.5	28.5	2.00	0.18	53	0.36
62.6	5425	4.0	28.5	1.88	0.24	59	0.36
66.7	7010	4.1	32.8	2.18	0.26	60	0.33
Yellowfin tuna (*Thunnus albacares*)							
46.2	1823	2.5	34.6	1.22	0.20	53	0.26
60.0	4056	3.5	29.9	1.48	0.22	53	0.42
64.4	5035	4.0	27.1	1.82	0.22	61	0.38
70.0	6497	4.0	28.8	2.02	0.22	52	0.43
80.6	9997	4.6	27.4	2.40	0.24	57	0.48
82.0	10537	4.5	27.1	2.24	0.24	60	0.42
147.9	65421	7.5	25.0	3.22	0.34	68	0.54
Bluefin tuna (*Thunnus thynnus*)							
200	272400	15.0	20.8	8.00	0.68	60	0.87*
Atlantic mackerel (*Scomber scombrus*)							
–	167.5	0.9	36.6	0.50	0.20	35	0.36
Dolphin (*Coryphaena hippurus*)							
120.3	–	3.5	26.0	1.82	0.20	0	1.82
Striped marlin (*Tetrapturus audax*)							
180.3	–	7.1	20.8	2.22	0.27	20	1.11
Wahoo (*Acanthocybium solanderi*)							
129.9	–	3.0	27.1	1.40	0.16	0	1.40
Swordfish (*Xiphias gladius*)							
183	107000	10.6	18.7	4.04	0.36	30	1.00
Atlantic cod (*Gadus morhua*)							
133	19000	3.4	9.5	2.40	0.86	0	2.40

Blood Pathway and Lamella Size

From each of 10 skipjack tuna, 7 yellowfin tuna, and 1 bluefin tuna, measurements of length, width, angle, and length of respiratory blood channels at the middle of the selected secondary lamella were made. These data are given in Table 1 for the 18 specimens of tunas, together with data for a few other species. Lamella length and width are plotted against fish weight (log–log) in Fig. 4 along with data for some other teleosts taken from the literature. The discrepancy between the present tuna data and those from Muir and Hughes (1969) is due to the fact that in the latter an effort was made to locate the longest lamella for each specimen, whereas in this study we simply selected a lamella from a standardized location. Those for other species from the literature are not specified but we assume that they are averages. The lines shown were fitted by eye and have slopes of approximately 0.28 for lamella length and 0.23 for width. The sum of these two exponents is 0.51, which compares well with 0.53 obtained by Muir and Hughes (1969) for the relation between area of an average lamella and tuna weight.

We note that many of the species measured by Hughes (1966) have relatively wide lamellae and thus, as he pointed out, have large lamellar areas. Exceptions acknowledged, different species of the same weight tend to have secondary lamellae of generally the same size. The great (22-fold) range in total lamellar area between species is largely accounted for by variation in total number of secondary lamellae, which is determined by the number per millimeter of filament and the total filament length.

Our interest at this point is mainly with lamella length and the data suggest a fairly consistent relation with weight for most species. It is sufficient for our present purpose to conclude that the very long lamellae of tuna (and other large fish) are unique only because the fish are large.

The length of the respiratory blood channel is plotted against lamellar length in Fig. 5. The straight line marked "other teleosts" has a slope of 1 and indicates that the channels run the full length of the lamellae. We observed this in some we examined and it is implied by other workers for a variety of teleosts. The length of the blood channel in the tunas is dramatically less. Mackerel, marlin, and swordfish are intermediate, showing some degree of obliqueness to the channels.

Length of the secondary lamellae varies over the gill arch for a single specimen. As lamella length and channel length are approximately the same for "other teleosts," all data for one specimen would fall on the indicated straight line. For the tunas, the oblique channels tend to be of consistent length, or vary only slightly over most of the range of lamella lengths for any specimen. In the tunas, therefore, data for one specimen essentially fall on the curved line representing specimens of different size.

Blood-Pressure Drop across Secondary Lamellae

With laminar flow, the Hagen-Poiseuille equation for pressure drop (Δp) in a tube (Daily and Harleman 1966 p. 118) is

$$\Delta p = \frac{32\mu V l}{d^2} \text{ dynes/cm}^2. \tag{1}$$

We assume that the blood channels are sufficiently cylindrical in cross section that the measured diameter (d) is also the equivalent diameter. μ is viscosity, V the mean velocity, and l the passage length.

From equation 1, we may derive an expression for total pressure drop through all the blood channels of the entire gill. Mean velocity is equal to the total volume flow (cardiac output) divided by the total passage area. Therefore,

$$V = \frac{Q}{n\frac{\pi d^2}{4}}, \tag{2}$$

where Q is cardiac output and n is the number of blood channels of diameter d in the entire gill.

Substituting equation 2 in 1

$$\Delta p = \frac{4(32\mu Q l)}{n\pi d^4}. \tag{3}$$

Especially in the tunas, the channel diameter (d) is remarkably uniform, on the average, over the whole gill so the number of channels (n) in equation 3 may be expressed as a function of gill surface area. The surface area overlying the blood channels (it being noted that there are two surfaces to a secondary lamella) is:

$$A_g = 2\,ndl \text{ or } n = \frac{A_g}{2dl}. \tag{4}$$

Substituting for n in equation 3 gives

$$\Delta p = \frac{256\mu Q l^2}{\pi A_g d^3} \text{ dynes/cm}^2, \tag{5}$$

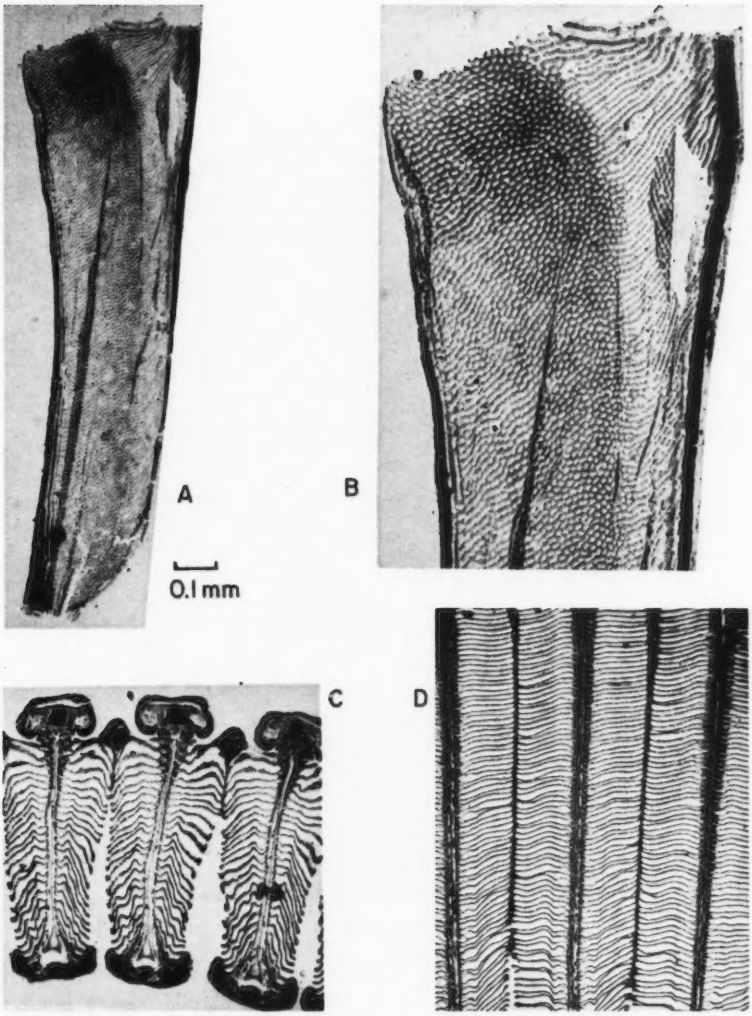

FIG. 2. Secondary lamellae of skipjack tuna: A, whole mount of single secondary lamella (see text); B, enlargement of above; C, thin section through three filaments, obliquely cutting many secondary lamellae. Note lamellar fusions; D, longitudinal section perpendicular to C showing three filaments and two lamellar fusions. Actual width of lamellae is approximately the same in all four photographs.

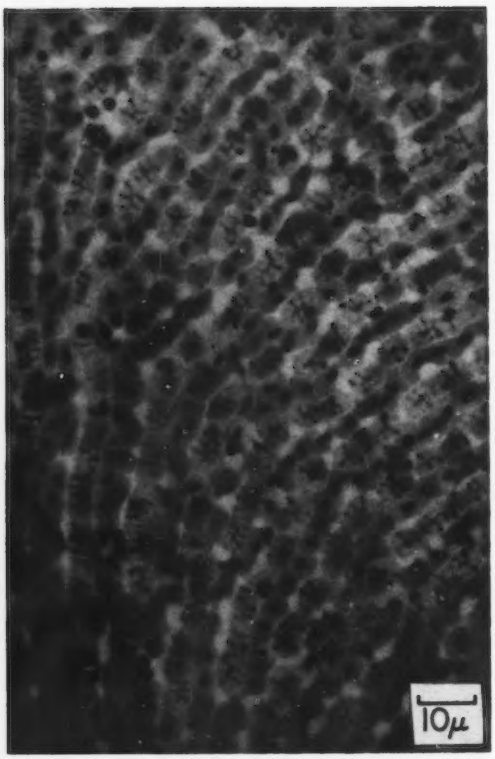

FIG. 3. Portion of whole mount of secondary lamella of bluefin tuna as in Fig. 2A and B.

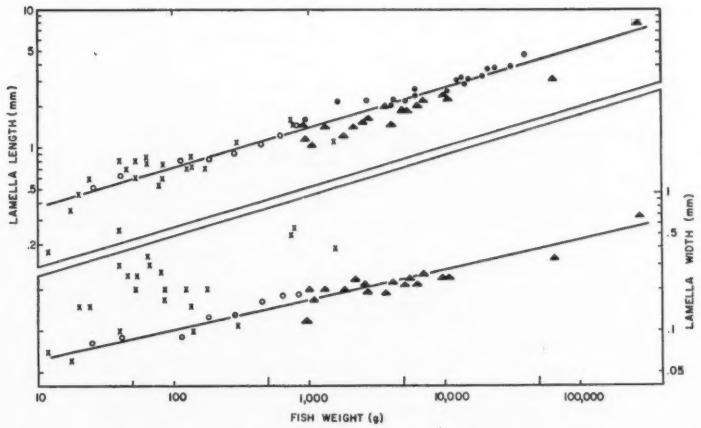

FIG. 4. Logarithmic plots of lamellar length and width against fish weight. ▲, Tunas from this study; ●, tunas from Muir and Hughes (1969); ○, bass (Price 1931); ×, various species reported by Hughes (1966). The measurements are not necessarily comparable among studies (see text).

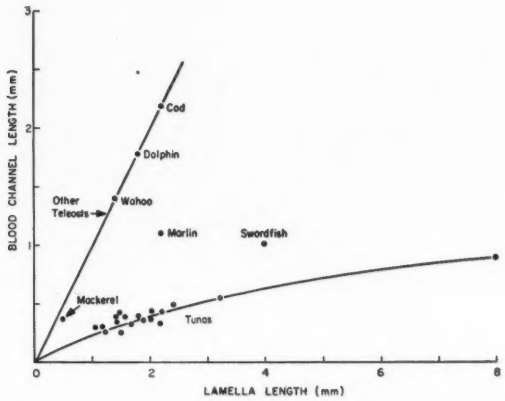

FIG. 5. Relation between length of secondary lamella and blood channel length. The line labelled "other teleosts" has a slope of 1.

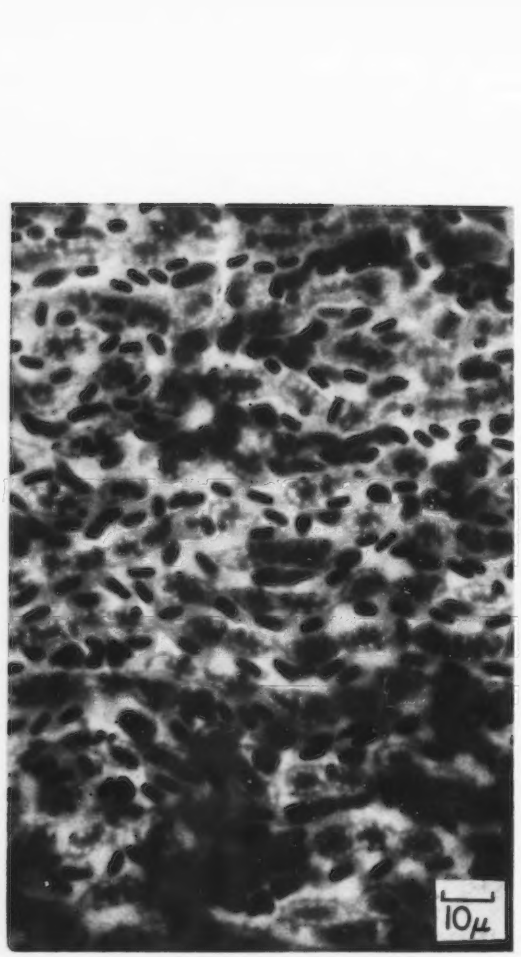

FIG. 6. Portion of whole mount of secondary lamella of Atlantic salmon as in Fig. 2 and 3.

which may be converted to millimeters Hg by multiplying by $760/10^6$.

Equation 5 is an expression for the total blood-pressure drop through all channels of the gill; it implies that the pressure drop increases as the square of the individual channel length. The oblique channels in the 272-kg bluefin were 0.09 cm long. Had the channels run the length of the lamella, as in other teleosts, they would have been 0.8 cm long and the pressure drop 79 times greater.

Precise measurements for all the parameters in equation 5 are lacking, but those available allow some instructive calculations to be made. We assigned a value of 0.024 poise for blood viscosity. Hematocrit and serum proteins vary with such things as species, sex, season, and size, so in reality the viscosity of fish blood may range widely. Special considerations such as the Fahraeus-Lindqvist effect of viscometer tube radius (Haynes 1960) are beyond the scope of this analysis. The effective gill area (A_g) for a 1.67-kg skipjack is taken as 65% of the measured area or 20,000 cm² (Brown and Muir 1970) and a reasonable calculated cardiac output is 1.8 ml/sec (Brown and Muir in preparation). For a skipjack tuna of this size (Table 1) the selected lamella was 0.142 cm long and the blood channel length was 0.034 cm. The average diameter of the blood channels was about 3.4×10^{-4} cm. For a first approximation, we take these values to represent the entire gill. In the tunas the length and diameter of the blood channels are notably uniform over most of the arch for a single specimen, even though the lamella length varies considerably, so the assumption is justified.

Putting these values into equation 5,

$$\Delta p = \frac{(256)(0.024)(1.8)(0.034^2)(760)}{(3.14)(2 \times 10^4)(0.00034^3)(10^6)} = 3.9 \text{ mm Hg},$$

which is low, but not out of line with measurements for some teleosts (Mott 1957). Had the channels followed the long axis (0.142 cm), the pressure drop would have been

$$\Delta p = 3.9 \times \frac{0.142^2}{0.034^2} = 68 \text{ mm Hg}.$$

Lacking other information, we have considered the 272-kg bluefin to be a large skipjack tuna and we assume the Q/A_g ratio remains fairly constant for a given species. (Gill area tends to increase with fish weight to a power of about 0.8 (Muir 1969; Muir and Hughes 1969), and cardiac output is linearly related to oxygen consumption (Garey 1970; Hanson and Johansen 1970), which also increases to the power of about 0.8). The bluefin tuna had a blood channel length of about 0.09 cm and a channel width of about 6×10^{-4} cm. The calculated blood-pressure drop is $\Delta p = 5$ mm Hg. Had the channels run the length of the lamellae (0.8 cm), the pressure drop would have been a preposterous 395 mm Hg.

The cardiac output used in these equations (1.8 ml/sec) was calculated for a skipjack swimming at its basal speed of 66 cm/sec (Brown and Muir in preparation). Cardiac output varies at least fourfold for the carp (Garey 1970) and sixfold for the dogfish (Hanson and Johansen 1970) and from equation 5 we see that pressure drop should vary directly with cardiac output. However, we do not know whether the pillar cells can function to offset this effect. Pressure drop varies as the cube of the channel diameter, so a small contraction of the pillar cells would result in a substantial change in blood-pressure drop.

Thus the absolute values calculated above lack precision, but the magnitude of the effect of the pathway on the blood-pressure drop leaves little doubt about the advantage of the modification in tuna. Indeed, it would seem that the large tunas could not cope with their tremendous gill dimensions if the basic teleost pathway was in use. Although tuna outrank most fish in size, other species do become large and must control the excessive blood pressure drop by other means.

Atlantic cod, for example, grow to at least 90 kg. Our 19-kg specimen had a lamella length of 0.24 cm and a blood channel width of about 6.2×10^{-4} cm for the selected lamella. For the cod (and trout and salmon) the channel length is approximately the same as the lamella length and this varies over the gill arch. The channel width also varies, however, so that l/d^2 (equation 1) tends to be somewhat constant for a specimen regardless of lamella size. Thus the value for the selected lamella can be used to represent the entire gill for a first approximation. It was considerably more difficult to obtain representative values for channel width in cod, trout, and salmon, because the pillar cells were not so neatly arranged as in the tunas and the spaces between were more variable (Fig. 6).

Cardiac output and gill surface area are smaller in the cod than in tuna but the Q/A_g ratio (required for equation 5) is not greatly different. From Saunders' (1963) data we have selected an oxygen consumption value of 115 mg O_2/hr at 15 C for a cod of about the same size as the 1.67-kg skipjack (Brown and Muir 1970), which consumed 700 mg/hr at 20 C. Cardiac output for cod is given as 9.3 ml/kg per min by Johansen (1962), which is about 0.26 ml/sec for 1.67 kg compared with about 1.8 ml/sec for skipjack (Brown and Muir in preparation). The congeneric *Gadus merlangus*

of 51 g has a total gill area of 217 cm^2 (Hughes 1966) compared with 1600 cm^2 for skipjack of the same size (Muir and Hughes 1969). Thus, with great qualification (see Discussion), we conclude that cod has an oxygen consumption of about 16%, a cardiac output of about 14%, and a gill area of about 14% of that for skipjack tuna.

The Q/A_g ratio for cod is sufficiently similar to that for tuna that we may use the tuna values for an order-of-magnitude calculation.

Substituting the cod values into the above calculations we get

$$\Delta p = 3.9 \times \frac{0.24^2}{0.034^2} \times \frac{0.00034^3}{0.00062^3} = 32 \text{ mm Hg.}$$

The gills from a smaller cod (about 1 or 2 kg) had a narrower channel width, about 4.5×10^{-4}. for the selected lamella. The calculated pressure drop for this fish was in the 20 mm range.

For a 260-g rainbow trout (*Salmo gairdneri*) the selected lamella was 0.06 cm long and the blood channels about 4.5×10^{-4} wide. We do not have gill area data for this species but the congeneric *Salmo trutta* of 175 g has a gill area of 593 cm^2 (Hughes 1966) compared with 4000 cm^2 for a skipjack of the same size (Muir and Hughes 1969). From Holeton and Randall (1967b) we obtain a cardiac output of about 2 ml/sec for a quiescent 1.67-kg rainbow trout (15 C; 520–1015 g), which is similar to the 1.8 calculated for that size skipjack (Brown and Muir in preparation). These values indicate that cardiac output is about the same, but gill area for the trout is only about 15% of that for tuna and the Q/A_g ratio for trout is about 6.5 times greater.

Substituting these values in the tuna equation we get

$$\Delta p = 3.9 \times 6.5 \times \frac{.06^2}{.034^2} \times \frac{.00034^3}{.00045^3} = 34 \text{ mm Hg.}$$

Holeton and Randall (1967a) measured a ventral aorta–dorsal aorta difference of about 18 mm Hg (9–16 C; no size range given). Stevens and Randall (1967b) give data from which we estimate a cardiac output of about 0.2 ml/sec for a 1.67-kg rainbow trout (5 C; 200–400 g). We calculate a pressure drop of 3.4 mm Hg compared with their measurement of 10 (Stevens and Randall 1967a).

The 7.6-kg Atlantic salmon (*Salmo salar*) had a channel width of 6.4×10^{-4} cm for the selected lamella, which was 0.2 cm long. The gill area was about 21,000 cm^2 compared with 110,000 cm^2 for skipjack of the same weight. If cardiac output is comparable to that of rainbow trout, then Q/A_g ratio for salmon is about 5.3 times that for tuna.

As above, we calculate:

$$\Delta p = 3.9 \times 5.3 \times \frac{.2^2}{.034^2} \times \frac{.00034^3}{.00065^3} = 102 \text{ mm Hg.}$$

This value is much larger than would be expected for Atlantic salmon and indicates the limited value of interspecies substitution of the required parameters. It may also indicate inadequacies in our sampling and measuring technique.

Despite the difficulty in determining the precise boundaries of the blood channels, our values show substantial differences with fish size for the three groups. These values are summarized in Table 2. A plot of the logarithm of diameter against the logarithm of fish weight (not shown) indicates a similar rate of increase for the three groups but a great difference in the intercept values.

Discussion

The above analysis considers the total drop in blood pressure across the secondary lamellae and ignores the contribution made by distributing and collecting vessels. The major portion of the resistance to flow is undoubtedly encountered in the secondary lamellae so most of the pressure loss between ventral and dorsal aortae should be included in our equation.

The values used for blood channel width are believed to be fairly representative but they are to be accepted with some qualification. The effect of formalin fixation was not determined but no obvious distortions were observed. The precise boundaries of the channels were difficult to locate and considerable variation in channel width was encountered over the field of a lamella. The measurements were verified by a colleague working independently, however, so they are at least reproducible. A potentially greater source of error may be associated with the poorly understood function of the pillar cells. The pillar cells appear to be modified smooth muscle cells (Munshi and Singh 1968); if they can modify blood channel width, then the blood-pressure drop could be substantially

TABLE 2. Summary of fish weights and lamellar blood channel diameters.

Species	Wt (*kg*)	Diam (μ)
Skipjack tuna	1.67	3.4
Bluefin tuna	277	6.0
Atlantic cod	1–2	4.5
Atlantic cod	19	6.2
Rainbow trout	0.26	4.5
Atlantic salmon	7.6	6.4

altered. If the diameter could increase from, say, 4×10^{-4} cm to 5×10^{-4} cm, then the pressure drop would be halved — just offsetting a doubling of cardiac output.

From the reports of Stevens and Randall (1967a, b) we note that during exercise the cardiac output increases three to five times (depending on the method of calculation) but that blood pressure increases only about two times. The experiments were done separately and, as the calculated values for cardiac output are inherently less reliable than the measured blood-pressure drop, we are allowed no certain deduction concerning changes in channel diameter. Comparison of the lamellae from rainbow trout that had been quickly pithed with those that had been subjected to extreme hypoxia gave no indication of a change in diameter. Nor did the comparison reveal any increase in blood cells in the channels. Properly designed experiments might provide more information.

The diameters we measured for this analysis may pertain to metabolic levels that are not at all comparable to those under which the other values were determined. Indeed, in view of the effect of environmental conditions and physiologic states on oxygen consumption and cardiac output, it is remarkable that so many of our calculated pressures are even of the right approximate magnitude.

The precise application of the model requires measurements that are simply not available for a single species. The approximate .application, however, by providing insight into the contribution to blood-pressure drop made by the various parameters and the magnitude of adjustments that might be required, allows some useful conclusions.

In all teleosts, increasing blood-pressure drop with increasing lamella size would appear to be a problem; most species may overcome it by increasing the diameter of the lamellar blood channels. Blood pressure itself increases with fish size (Greene 1905) but there is little information on whether much of the increase is absorbed in the gill.

In tunas, and to some extent marlins and swordfish, the problem has been met by shortening the length of the small diameter respiratory vessels. For the large bluefin tuna to operate without its modification might require a reduction of the blood pressure drop from 395 mm Hg to, say, 40 mm Hg. This could be accomplished by a 10-fold increase in its cube of the vessel diameter (equation 5) or a doubling of the diameter to about 13 μ. The result would be a reduction of the number of blood channels in the same gill area and probably require an increase in the thickness of the epithelial layer and in the pillar cell size to support the larger channels. This would decrease the efficiency of gas exchange. In addition, the resulting increase in secondary lamellar thickness would encroach upon the water channel thickness and increase the resistance to water flow.

Undoubtedly the observed geometry represents an adequate, if not the best, solution consistent with the organism–environment interaction peculiar to the tunas. Whereas some teleosts, as pointed out by Alexander (1967), do not seem to have developed as large a gill surface area as possible, the tunas certainly seem to have done so. General consideration of gill geometry permits the working hypothesis that the combination of a large streamlined body, large relative gill surface area, and high metabolic activity is not possible without changes in the basic teleost gill design. Our present support for the hypothesis, outlined in the following discussion, concerns only the cost of moving blood and water. Water and ion balance must be important also, but we have not yet included this. This omission from our present discussion may not be serious, for we have not tampered greatly with the blood and water flows or exposure times.

The volume and configuration of the space allotted to the gill apparatus are the result of the interaction of several systems and functions, some of which, of course, are associated with the gill itself. To simplify, we may consider that the space is fixed and that the only consideration is to efficiently fit the required gill surface area into it. The available space places limits on the number and length of the secondary lamella-bearing filaments and on the length of secondary lamellae. Width of the secondary lamellae is also fixed through interaction with the number of filaments (it occupies the interfilament space). Spacing of the secondary lamellae may be independent of head size per se, but its hydrodynamic interaction with lamellar length gives it an indirect dependence.

The large gill area of the tunas results from a relative increase in the total number of secondary lamellae, the size of which is not large in relation to other species of the same size. The increase in number is achieved by an increase in the number and length of filaments and by an increase in the number of lamellae per unit length of filament (spacing). In all teleosts, increasing fish size results in an increase in the length and spacing of the secondary lamellae (Muir 1969).

From equation 1 the pressure drop in a single passageway for both water and blood is proportional to the velocity and the passage length and to the inverse of the square of the passage width

$$\Delta p \propto \frac{Vl}{d^2}.$$

With respect to the blood, as discussed above, increasing channel length requires a compensating increase in the diameter. For the large bluefin tuna the channel diameter is about 6 μ and the lamellar thickness about 10 μ.[2] Without the modified pathway the diameter would have had to be 13 μ and the total lamellar thickness might be about 20 μ. The water passage thickness is about 40 μ (20 lamellae/mm), but if the lamellar thickness increased to 20 μ this would be reduced to 30 μ. It follows that water pressure drop would increase 2.34 times – it would increase by $40^2/30^2$ because of the change in thickness (d) and by 4/3 because of increased velocity required to get the same quantity of water through a reduced passage area. Magnuson (1970, and personal communication) has shown that basal swimming speed for the small tuna species is determined by buoyancy requirements. At the basal speeds calculated by Magnuson, Brown and Muir (1970) calculated that a 1.67-kg skipjack is already using half of the available pressure head. An increase in swimming speed might be required for ventilation if the water pressure drop were greatly increased. In addition to raising the metabolic level this would make ventilation the main factor determining basal swimming speed.

An alternative solution would involve a sacrifice in gill area to increase the water passage thickness, but this is not the line followed by the tunas. The specialized blood pathway of the tunas serves to prevent excessive drop in both blood pressure and water pressure and allows retention of relatively large gill surface area.

Further consideration of our hypothesis concerning fish size and activity must await observations on large nontunas. Regardless of the strength of that hypothesis, the present understanding of the significance of and interrelation between the geometry of the blood and water pathways may be of substantial value in deducing the gill design criteria significant to other species.

Acknowledgments

We are greatly indebted to N. Brady for her patience and skill in microdissection. Mr N. Prouse verified the measurements of blood channel dimensions without prior knowledge of their intended use. J. C. MacKinnon, D. J. Randall, and J. C. Smith offered helpful advice on the manuscript.

[2]Our earlier (Muir and Hughes 1969) value of 10 μ for skipjack was measured at the free border of the secondary lamella. This border is thickened by the presence of the larger feeder vessels (Fig. 2) and a more appropriate value for skipjack is 6 μ. Thus the water passage thickness may be more like 24 μ than 20 and the total water passage area used by Brown and Muir (1970) an underestimate.

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Vulnerability of Some Fishes to Northern Pike (*Esox lucius*) Predation[1]

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MAUCK, W. L., AND D. W. COBLE. 1971. Vulnerability of some fishes to northern pike (*Esox lucius*) predation. J. Fish. Res. Bd. Canada 28: 957–969.

To compare vulnerability to predation by northern pike (*Esox lucius* Linnaeus), several species of fish in various combinations were held with pike in plastic swimming pools with and without cover and in small ponds. The most vulnerable species in order were gizzard shad (*Dorosoma cepedianum*), carp (*Cyprinus carpio*), bigmouth buffalo (*Ictiobus cyprinellus*), fathead minnow (*Pimephales promelas*), and smallmouth bass (*Micropterus dolomieui*). White sucker (*Catostomus commersoni*), green sunfish (*Lepomis cyanellus*), largemouth bass (*Micropterus salmoides*), golden shiner (*Notemigonus crysoleucas*), and yellow perch (*Perca flavescens*) showed intermediate vulnerability. Channel catfish (*Ictalurus punctatus*), northern pike, bluegill (*Lepomis macrochirus*), and black bullhead (*Ictalurus melas*) were least vulnerable. In two experiments in which fish were put in cages in turbid water, relative vulnerability was the same as in clearer water. Pike could not be conditioned to eat golden shiner or bluegill. There was a tendency for certain sizes of pike to select the smaller carp, fathead minnow, and bluegill within the size ranges tested.

MAUCK, W. L., AND D. W. COBLE. 1971. Vulnerability of some fishes to northern pike (*Esox lucius*) predation. J. Fish. Res. Bd. Canada 28: 957–969.

Dans le but de comparer les degrés de rapacité du brochet (*Esox lucius* Linnaeus), on a placé celui-ci avec plusieurs espèces de poissons préalablement combinées, dans des bassins de plastique avec ou sans abri et dans de petits étangs. Par ordre décroissant, la rapacité du brochet s'est manifestée surtoût envers le *Dorosoma cepedianum*, puis envers le *Cyprinus carpio*, l'*Ictiobus cyprinellus* et enfin envers le *Pimephales promelas*. Sa rapacité était moyenne à l'égard du *Micropterus dolomieui*, du *Catostomus commersoni*, du *Lepomis cyanellus*, du *Micropterus salmoides*, du *Notemigonus crysoleucas* et de la *Perca flavescens*. La rapacité était moindre envers l'*Ictalurus punctatus*, l'*E. lucius*, le *Lepomis macrochirus* et l'*Ictalurus melas*. Lors de deux expériences où les poissons ont été mis en cage en eau trouble, la rapacité relative s'est avérée la même qu'en eau claire. Le brochet n'a pu être conditionné à manger ni le *N. crysoleucas* ni le *L. macrochirus*. Des brochets de certaine taille avaient tendance à choisir, dans la série de tailles examinées, les *C. carpio*, les *P. promelas* et les *L. macrochirus* de taille inférieure.

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THE objective of this study was to compare vulnerability of various fishes to predation by northern pike, *Esox lucius* Linnaeus. In several experiments, known numbers of fishes were placed in pools and ponds with northern pike, and the pike were allowed to feed until approximately 25% of the prey had been consumed. In general, the "nongame" fishes were found to be more vulnerable to predation than "game" fishes. Many investigators have found fishes to be selective in their feeding (e.g., Kutkuhn 1957; Ivlev 1961; Brooks and Dodson 1965; Galbraith 1967; Carothers and Allison 1968; Parsons and LeBrasseur 1970). Studies that indicated that northern pike are selective feeders include

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those of Beyerle and Williams (1968), Allen (1939), Hunt (1965), and Johnson (1969).

Vulnerability may be thought of as a function of two factors operating simultaneously: availability and preference. Availability (accessibility: Ivlev 1961) is a function of prey, and preference (predilection: Ivlev 1961) is a function of predators. Vulnerability may be taken to mean being subject to attack, and availability as capable of being made use of (Funk and Wagnalls 1940). Availability of prey may be influenced by their appearance and behavior, armament, size, distribution, and relative abundance. Preference may be defined as a propensity of a predator to consume one size or species of food rather than another. Preference of predators may be affected by their innate inclination, satiation, and conditioning.

Methods and Materials

Most experiments were performed in plastic swimming pools and ponds at the Fish-Pesticide Research Laboratory (U.S. Bureau of Sport Fisheries and Wildlife) near Columbia, Missouri (38°55′N, 92°20′W). Experiments were conducted from June through September in 1968 and 1969. Northern pike were obtained from the U.S. Bureau of Sport Fisheries and Wildlife's National Fish Hatchery at Guttenberg, Iowa, and from the National Fish Hatchery at Valley City, North Dakota, in 1968 and 1969. The pike obtained from Valley City in 1968 had been fed only fathead minnow (*Pimephales promelas*) at the hatchery and those obtained in 1969 had not been fed fish at all. Pike from Guttenberg had been fed fathead minnow and other minnows and goldfish (*Carassius auratus*).

During the study pike were held in pools, or a pond in the case of five pike larger than 443 mm, when not used in an experiment. Between experiments, all northern pike were fed goldfish except for those obtained from Valley City in 1969 (used in experiments 1–4); they were fed fathead minnow because they were to small for the available goldfish. Four size ranges of pike used in the two summers were: 110–168 and 224–450 mm in pool experiments, 224–509 and 443–1010 mm in pond experiments.

Prey fishes, obtained from national and private fish hatcheries and from wild populations, were held in portable plastic swimming pools. In data presented in tables, many common names of fishes are abbreviated. Common and scientific names of fishes used in the study, as well as the abbreviations, are:

Scientific name	Common name	Abbreviation
Dorosoma cepedianum	Gizzard shad	Shad
Cyprinus carpio	Carp	Carp
Ictiobus cyprinellus	Bigmouth buffalo	Buff
Pimephales promelas	Fathead minnow	Fathd
Notemigonus crysoleucas	Golden shiner	Shiner
Catostomus commersoni	White sucker	Sucker
Perca flavescens	Yellow perch	Perch
Lepomis cyanellus	Green sunfish	Green
Micropterus dolomieui	Smallmouth bass	SMB
Micropterus salmoides	Largemouth bass	LMB
Lepomis macrochirus	Bluegill	BG
Pomoxis annularis	White crappie	W.Crap
Pomoxis nigromaculatus	Black crappie	B.Crap
Esox lucius	Northern pike	Pike
Ictalurus melas	Black bullhead	Bullhd
Ictalurus punctatus	Channel catfish	C.Cat
Carassius auratus	Goldfish	G.Fish

Pools

The circular plastic swimming pools were 3.05 m (10 ft) in diameter and were filled to a depth of 51 cm (20 inches) with a continuous flow (3.4 liters/min) of aerated well water. A canopy of fiberglass netting shaded the pools. Water temperature ranged from 24 to 15 C during the two summers with a diel variation of approximately 2.8 C. A wire screen cover was used on holding pools containing northern pike to prevent them from jumping out, but not on experimental pools.

Experiments were conducted in pools with and without cover. Limbs of shingle oak (*Quercus imbricaria*) placed in the pools provided cover from top to bottom in the water column and covered about 30% of the surface area.

Ponds

The ponds were slightly less than 0.04 ha (0.1 acre) in area. Vegetation along the edges of the clay basins included some scattered *Sagittaria* in the water and bluegrass mixture on the banks extending into the water, both providing some cover for pike and prey. Maximum depth ranged from 65 to 122 cm. Summer water temperatures ranged from 29 to 17 C at the surface and from 28 to 17 C at the bottom. Water was always transparent; a Secchi disc could be seen at the maximum depth of all ponds.

Cages

Two turbidity experiments were conducted in cages covered with 6.35 mm (¼-inch) mesh hardware cloth. Cages were 3 × 0.75 × 0.9 m. They were placed in turbid and clearer ponds with combinations of prey fishes and pike to investigate effects of turbidity on vulnerability. These ponds were in the University of Missouri's Ashland Wildlife Research Area (38°48′N, 91°51′W), approximately 28.9 km southeast of Columbia, Mo.

PROCEDURE

For investigating species selection, 26 experiments were conducted in plastic pools, 15 in ponds, and 2 in cages. In an experiment, equal numbers of prey fishes (from 10 to 30 of each species) were put into a pool, pond, or cage. Combinations included three to eight species of prey fish. Usually prey were measured and put in the pools, ponds, or cages 0.5–1.5 hr before northern pike were introduced. Prey fishes of similar size were used in each experiment so that any differences in vulnerability could be attributed to selection of species rather than of size of prey (Tables 1–3). When slender-body fish, such as yellow perch, were offered with heavier-body fish, such as green sunfish, perch about 10 mm longer than green sunfish were used. Such differences in size between species varied with size of prey.

Information on size selection was obtained from some of the species-selection experiments and also from four experiments in which a single species of prey was put in pools with northern pike. Fathead minnow were used as prey in two of the latter experiments, and golden shiner and bluegill in the remainder. Selection was determined by comparing the length-frequency distribution of prey eaten to that of prey offered. Such analysis is complicated when more than one species is present, for then selection may be influenced by both species and size. In spite of the complication, length-frequency distributions were compared for carp, golden shiner, bigmouth buffalo, and bluegill eaten and offered in experiments 15–24. For those species there was a reasonably large range of sizes available, and in those experiments the pike were of similar size and the prey could be identified from their lengths at the end as well as the beginning of an experiment. In some of the other pool experiments (numbers 1–14) the range of sizes of prey was small, and in the pond experiments (numbers 27–41), which extended for up to 9.5 days, growth of prey was so variable that it was not possible to match lengths at the end of an experiment with those at the beginning to determine which fish had been eaten.

Each experiment involved one control pool, pond, or cage containing only prey, and two to five other pools, ponds, or cages containing pike as well as prey; that is, two to five replicates. Usually three or four pike were placed in each pool, five to eight in a pond, and two in each cage. Pike were dipped from a holding pool containing 25–250 pike depending on size. Experiments ended when fish were removed, counted, and measured. Pike were returned to the holding pool. Pool experiments were stopped after about 25% of the prey were eaten. Pond experiments ran from 5 to 9.5 days, a period in which 7–45% of prey was eaten. At the end of a pond experiment, fish were removed by seining; then the ponds were pumped down, and any remaining fish were removed before ponds were refilled with aerated well water for the next set of experiments.

Differences in vulnerability of various prey species were expressed quantitatively by means of the electivity index (E) of Ivlev (1961):

$$E = (r - p)/(r + p),$$

where r is the relative content of a species in the ration, and p is the relative content of the same species in the environment. Values of E range from −1.0 to +1.0. A positive value indicates that fish were eaten in greater proportion than they were present in the environment; a value of zero, that fish were eaten in the same proportion that they were present in the environment; and a negative value, that fish were eaten in a smaller proportion.

Chi-square values were calculated to determine if observed differences in vulnerability were significant. An IBM Model 360 computer was used to calculate chi-square values testing the hypothesis that ratios of eaten to not-eaten fish were independent of the species of prey. Results of replicates in the experiments were so similar that data were pooled for each experiment.[5]

Species Selection

Relative vulnerability of prey to northern pike predation was the same for all sizes of pike in pools without cover (an unnatural environment), pools with cover (a more natural environment), and ponds (a nearly natural environment). Gizzard shad were most vulnerable, followed in order by goldfish (used in two experiments only), carp, bigmouth buffalo, fathead minnow, and smallmouth bass (Fig. 1). White sucker, green sunfish, largemouth bass, golden shiner, and yellow perch showed intermediate vulnerability, and channel catfish, northern pike, bluegill, and black bullhead were least vulnerable. The curve in Fig. 1 seems to be composed of three segments separated by inflections between channel catfish and yellow perch and between white sucker and smallmouth bass. Differences in percentage eaten between adjoining species at the points of inflection are significant at $P = 0.10$.

Exceptions to these generalizations were apparent in some experiments. Variation in results seemed to be related to the combination of prey present and to factors influencing availability, such as size, armament, and disease of prey.

Relative vulnerability of prey in pools did not seem to be influenced by cover (Tables 4, 5). Species showed high, intermediate, or low vulnerability regardless of cover. Disease appeared to increase vulnerability of prey. Vulnerability of smallmouth bass increased throughout experiments 6–14 (Table 4), when smallmouth bass developed symptoms of *Columnaris* disease. The increase in vulnerability was correlated with mortalities observed in both the control (mortality increasing from 0 to 90%) and experimental pools. White crappie was the

[5]For results of each replicate of each experiment as well as dates and water temperature for each, see Mauck (MS 1970).

TABLE 1. Median lengths (mm), and (in parentheses) ranges, of prey fishes offered to pike 110–168 mm long in pools, 1968 and 1969. Prey species were not measured in experiments 5, 6, 8, 9, 10, and 11, but were similar in size to those used in experiment 7.

Expt. no.	Shad	Carp	Fathd	Shiner	Sucker	Perch	SMB	LMB	B.Crap	Green	BG	Bullhd
1	32(30–35)	–	–	–	–	47(43–53)	–	37(33–43)	–	38(35–47)	37(32–48)	30(25–36)
2	35(33–40)	–	–	–	–	45(41–49)	–	40(37–45)	–	38(34–42)	36(30–43)	30(28–36)
3	42(34–45)	–	45(40–53)	–	–	45(42–53)	–	39(35–43)	–	37(34–45)	36(32–45)	31(26–33)
4	–	48(44–54)	46(44–50)	57(55–61)	–	50(42–52)	–	–	47(42–48)	–	34(31–35)	34(33–37)
7	–	39(34–48)	46(42–50)	47(42–53)	47(44–54)	–	35(31–41)	32(28–37)	–	–	–	–
12	–	42(35–53)	44(40–48)	48(39–55)	–	–	40(33–45)	–	–	–	–	45(37–57)
13	–	40(34–52)	46(37–52)	47(39–52)	51(41–58)	–	38(32–45)	–	–	–	–	43(38–49)
14	–	41(36–50)	46(39–53)	47(35–53)	50(39–56)	–	40(34–44)	–	–	–	–	45(37–51)

TABLE 2. Median lengths (mm), and (in parentheses) ranges, of prey fishes offered to pike 224–450 mm long in pools, 1968 and 1969. Goldfish, similar in size to the bigmouth buffalo, were used in experiment 17 but were not measured.

Expt. no.	Shad	Carp	Buff	Fathd	Shiner	Perch	LMB	SMB	Green	BG	W.Crap	C.Cat	Bullhd
15	–	–	51(37–61)	–	63(48–75)	–	48(40–53)	–	–	–	58(47–63)	57(52–63)	45(38–51)
16	–	–	53(43–65)	–	64(50–100)	–	47(40–51)	–	–	–	59(53–62)	56(48–63)	44(41–50)
17	–	44[a](36–126)	–	45(41–51)	64(39–101)	–	–	–	–	–	58(47–61)	–	46(37–51)
18	–	–	50(44–60)	53(48–60)	65(50–120)	–	43(39–48)	–	–	–	58(47–64)	57(50–62)	–
19	–	48 (44–54)	–	55(52–60)	–	–	48(42–51)	–	56(47–70)	69(55–82)	–	58(53–61)	47(39–54)
20	–	116[a](52–129)	–	–	83(64–104)	–	61(55–65)	–	–	63(50–83)	–	–	–
21	–	–	–	–	97(63–110)	–	62(52–67)	–	57(46–67)	73(55–87)	–	58(54–82)	48(43–53)
22	–	49[a](44–120)	–	–	94(63–110)	–	61(52–67)	–	56(46–80)	73(55–89)	–	58(54–95)	–
23	–	108[a](42–127)	56(47–85)	56(51–63)	89(67–115)	–	60(52–64)	–	63(47–77)	–	–	–	50(43–58)
24	–	–	53(48–59)	–	99(63–120)	–	63(58–70)	67(59–73)	–	–	–	60(55–64)	–
25	–	–	–	–	92(80–111)	90(71–108)	77(68–86)	–	73(48–91)	70(48–89)	–	–	–
26	82(69–108)	76 (70–88)	–	–	93(85–107)	92(82–107)	–	81(70–92)	–	83(66–93)	–	–	–

[a]Median length of carp after those over 110 mm long were omitted in experiments 17, 20, 22, and 23 was 42, 52, 49, and 48 mm respectively.

TABLE 3. Median lengths (mm), and (in parentheses) ranges, of prey fishes offered to pike in ponds, 1968 and 1969. Experiments 27–36, pike 420–509 mm; experiment 37, pike 443–1010 mm; experiments 38–41, pike 224–316 mm.

Expt. no.	Shad	Carp	Buff	Fathd	Shiner	Perch	Green	SMB	LMB	W.Crap	BG	Pike	C.Cat	Bullhd
27	–	–	–	–	97(80–126)	93(72–126)	75(56–93)	–	82(71–97)	–	75(54–95)	99(83–112)	–	–
28	–	–	75(60–115)	72(69–86)	–	91(75–113)	–	–	–	–	72(58–89)	–	–	–
29	–	–	77(60–104)	75(70–84)	–	98(83–113)	–	–	–	–	74(55–99)	–	–	–
30	–	–	82(61–120)	–	91(74–130)	94(79–108)	78(57–108)	–	77(69–84)	–	73(55–90)	–	–	–
31	–	–	–	–	93(82–127)	94(74–116)	75(57–93)	–	81(68–97)	–	73(56–102)	–	–	–
32	–	–	–	–	96(82–127)	93(77–107)	73(55–89)	78(69–85)	80(73–87)	–	75(56–91)	–	–	–
33	90(75–115)	–	–	–	92(78–130)	99(82–112)	75(50–107)	–	77(69–85)	–	74(54–90)	–	–	–
34	89(70–111)	89(73–115)	–	–	104(83–117)	105(84–128)	–	80(73–89)	–	–	83(67–103)	–	–	–
35	140(110–185)	–	–	–	101(82–118)	98(80–114)	79(65–94)	83(75–94)	–	–	78(62–90)	–	–	–
36	93(76–105)	91(71–110)	–	–	98(83–116)	103(83–120)	–	83(74–94)	–	–	83(67–95)	–	–	–
37	140(98–192)	–	–	–	100(85–123)	99(85–117)	75(57–92)	85(74–100)	89(71–99)	–	77(59–96)	–	–	160(150–173)
38	–	44[a](37–123)	51(44–79)	–	63(46–75)	–	–	–	–	58(53–72)	–	–	–	–
39	–	120[a](80–131)	–	–	98(65–116)	–	–	–	64(55–78)	–	73(55–90)	–	–	–
40	–	119[a](87–126)	–	57(54–70)	98(67–127)	–	–	–	60(51–62)	–	73(56–88)	–	–	–
41	–	–	55(50–59)	–	101(65–130)	–	–	66(62–73)	66(60–72)	–	–	–	60(57–65)	–

[a]Median lengths of carp after those over 110 mm long were omitted in experiments 38, 39, and 40 were 43, 93, and 92 mm respectively.

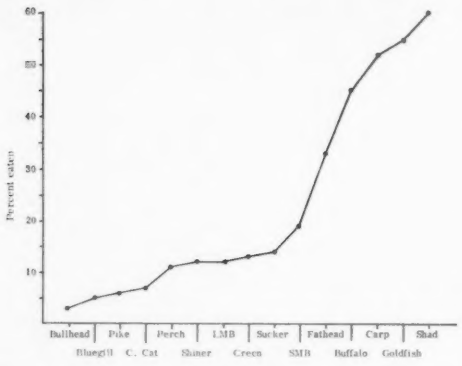

FIG. 1. Percentages of prey species that were eaten when offered to northern pike. Data for pools, ponds, and all sizes of pike combined. Data omitted from the comparison, explained in the text, include smallmouth bass showing symptoms of *Columnaris* disease, white crappie (which were emaciated), carp longer than 110 mm, spine-clipped black bullhead, and black crappie (used in only one experiment).

only other species exhibiting symptoms of disease during the study. They were emaciated and sluggish; hence, their vulnerability may have been erroneously high (Herting and Witt 1967).

Vulnerability of carp was influenced by their size in both pools (Table 5) and ponds (Table 6). Carp larger than 110 mm long were used in several experiments. As shown in the next section, such carp were not vulnerable to the pike. In experiments in which carp longer than 110 mm were used, the original electivity indices were corrected by omitting larger carp from consideration (Tables 5, 6).

Results were influenced by the combination of prey presented in experiments. Species that showed low or intermediate vulnerability when offered in experiments with more favored species of prey such as gizzard shad or carp exhibited higher vulnerability when presented without the more favored prey. For example, vulnerability of green sunfish was high in experiment 21, when the combination of species did not include gizzard shad, carp, bigmouth buffalo, or fathead minnow (Table 5). In experiment 22, with carp present, vulnerability of green sunfish was low.

Spines of ictalurids probably reduced their vulnerability to the predator. Black bullhead were more vulnerable in experiment 23, the only experiment in which their dorsal and pectoral spines were clipped, than in any of the other experiments in which the species was used. Only 18 black bullhead were eaten out of 455 offered during the study, and six of them were eaten in experiment 23. Other evidence that spines of prey provide protection against predators has been provided by Mossman (MS 1955) for black bullhead and yellow bullhead (*Ictalurus natalis*) and by Hoogland et al. (1956) for sticklebacks of the genera *Gasterosteus* and *Pygosteus*.

Turbidity had no clear effect on species selection. In the two experiments in a turbid and a clearer pond, pike predation was not clearly affected by turbidity. Gizzard shad and carp were most vulnerable, smallmouth bass, golden shiner, and yellow perch were intermediate, and bluegill were least vulnerable, regardless of turbidity (Table 7). Though gizzard shad and smallmouth bass tended to be more vulnerable in turbid than in clearer water, the limited data are inconclusive.

Conditioning appeared to have no effect on food selection by northern pike. Conditioning can be thought of as the training of an organism to feed on a specific food in the presence of a choice of foods. In two experiments in pools, six pike were fed only golden shiner for 65 and 114 days in an attempt to condition them to feed on golden shiner. Then the pike were offered a choice of food, and their diet was compared with that of pike that were offered the same choice of food but had not been fed golden shiner. The percentage of the ration made up of golden shiner was 12 for both groups of pike (Table 8). In another experiment, five pike were fed only bluegill for 72 days. When the pike were then offered largemouth bass, yellow perch, golden shiner, and bluegill, they ate just one bluegill in comparison with eight other fish.

Size Selection

Selection of prey on the basis of size was investigated by comparing length-frequency distributions of prey of five species presented to and eaten by northern pike. In the ranges of sizes of predator and prey used, pike tended to eat the smaller fathead minnow, carp, and bluegill, but did not seem to select any sizes of golden shiner or bigmouth buffalo (Fig. 2). Cover did not appear to affect size selection.

Pike less than 165 mm selected fathead minnow less than 50 mm long over larger minnows up to 90 mm long (Fig. 2A, B). No data from the species-selection experiments (Tables 4–6) were corrected for size of fathead minnow, however. In six of the experiments in which fathead minnow were used, the minnows were almost exclusively within the vulnerable size range, and in six other experiments in which larger fathead minnow were presented,

TABLE 4. Electivity indices of prey fishes to northern pike 110–168 mm long in pools with and without cover, 1968 and 1969.

Expt. no.	Shad	Carp	Fathd	Shiner	Sucker	Perch	SMB	LMB	B.Crap	Green	BG	Bullhd	G.Fish	Total No. offered	Total No. eaten
							With cover, 1969								
1	+.714[a]	–	–	–	–	−1.000	–	−1.000	–	−1.000	−1.000	−1.000	–	120	18
2	+.589[a]	–	–	–	–	+.072	–	−.128	–	−1.000	−.678	−1.000	–	120	31
3	+.640[a]	–	−.243	–	–	−.243	–	+.098	–	−1.000	−1.000	−1.000	–	140	23
4	–	+.533[a]	+.210	−.067	–	−.644	–	–	−.388	–	−.388	−.644	–	140	32
							Without cover, 1968								
5	–	–	+.227	–	−.062	–	–	−.308	–	–	–	–	–	60	17
6	–	–	+.184	–	−1.000	–	−.160	–	–	–	–	–	+.289	80	11
7	–	+.287[a]	+.026	−.095	−.377	–	−.140	+.059	–	–	–	–	–	180	80
8	–	+.370	+.092	−.724	−.351	–	−.017	–	–	–	–	–	–	200	62
9	–	+.249[a]	+.148[b]	−.449	−.526	–	−.033[b]	–	–	–	–	–	–	200	48
10	–	+.361	−.010	−.616	−.101	–	−.101	–	–	–	–	–	–	200	61
11	–	+.333[a]	+.152[a]	−.694	–	–	+.152[a]	–	–	–	–	−.834	–	200	55
12	–	+.263	+.304	−1.000	–	–	+.168	–	–	–	–	−1.000	–	150	32
13	–	+.298[a]	+.105[a]	−.529	−.529	–	+.396[b]	–	–	–	–	−1.000	–	240	39
14	–	−.104	+.419	−.456	−.301	–	+.059	–	–	–	–	−.456	–	240	32
												Total		2270	541

[a,b]For each experiment, E values marked with a superscript are significantly different (chi-square test; $P = 0.05$) from any other E value not having the same superscript. For statistical analysis, data were combined for experiments 8–10; 11 and 12; 13 and 14.

TABLE 5. Electivity indices of prey fishes to northern pike 224–450 mm long in pools with and without cover, 1968 and 1969. Values in parentheses are corrected. The correction, explained in text, consisted of recalculating electivity values after omitting data for carp over 110 mm long. Carp in other experiments were less than 110 mm long.

Expt. no.	Shad	Carp	Buff	Fathd	Shiner	Perch	LMB	SMB	Green	BG	W.Crap	C.Cat	Bullhd	G.Fish	Total No. offered	Total No. eaten
							Without cover									
15	–	–	+.201[a]	–	–.301	–	–.456	–	–	–	+.501[a]	–.685	–1.000	–	240	32
16	–	–	+.384	–	–.281	–	–.566	–	–	–	+.408	–.566	–.412	–	240	43
17	–	+.305[a]	–	–.194[b]	–.635	–	–	–	–	–	–.095[b]	–	–.537	+.356[a]	240	80
	–	(+.438)	–	(–.219)	(–.651)	–	–	–	–	–	(–.122)	–	(–.555)	(+.333)	(228)	(80)
18	–	–	+.231[a]	+.231[a]	–.668	–	–.165	–	–	–	+.231[a]	–.668	–	–	240	30
19	–	+.586[a]	–	–.219	–	–	–.122	–	–.219	–.219	–	–1.000	–.515	–	105	23
20	–	–.030	–	–	+.243	–	–.362	–	–	–.030	–	–	–	–	60	17
	–	(+.400)	–	–	(+.156)	–	(–.438)	–	–	(–.120)	–	–	–	–	(50)	(17)
21	–	–	–	–	–.077	–	–.077	–	+.549[a]	–.277	–	–.558	–1.000	–	240	21
							With cover									
22	–	+.573[a]	–	–	–.021[b]	–	–.099[b]	–	–.573	–.756	–	–.756	–	–	180	44
		(+.600)	–	–	(–.030)	–	(–.108)	–	(–.579)	(–.760)	–	(–.760)	–	–	(177)	(44)
23	–	+.159[a]	+.544[b]	+.003[a]	–.604	–	–.604	–	–1.000	–	–	–	–.140[a]	–	210	56
	–	(+.436)	(+.516)	(–.030)	(–.621)	–	(–.621)	–	(–1.000)	–	–	–	(–.180)	–	(195)	(56)
24	–	–	+.552[a]	–	–.284	–	–.568	–.183	–	–	–	–1.000	–	–	150	36
25	–	–	–	–	–.090	+.538[a]	–.286	–	–1.000	–.563	–	–	–	–	150	18
26	+.411	+.411	–	–	–.427	–.133	–	–1.000	–	–1.000	–	–	–	–	120	15
														Total	2175	415

[a,b]See Table 4. For statistical analysis, data were combined for experiments 15 and 16.

TABLE 6. Electivity indices of prey fishes to northern pike in ponds. Experiments 27–36, pike 420–509 mm; experiment 37, pike 443–1010 mm; experiments 38–41, pike 224–316 mm, 1968 and 1969. Values in parentheses are corrected. The correction explained in text consisted of recalculating electivity values after omitting data for carp over 110 mm long. Carp in other experiments were less than 110 mm long.

Expt. no.	Shad	Carp	Buff	Fathd	Shiner	Perch	Green	SMB	LMB	W.Crap	BG	Pike	C.Cat	Bullhd	Total No. offer	Total No. eaten
27	–	–	–	–	+.199	–.053	+.090	–	–.053	–	–.251	–.053	–	–	300	20
28	–	–	+.343[a]	+.108[b]	–	–.170	–	–	–	–	–1.000	–	–	–	240	45
29	–	–	–.022	+.415	–	–.634	–	–	–	–	–.436	–	–	–	240	71
30	–	–	+.223[a]	–	–.518	+.023	+.308[a]	–	–.309	–	–.227	–	–	–	300	57
31	–	–	–	–	–.149	–.052	+.249	–	–.052	–	–.460	–	–	–	300	27
32	–	–	–	–	+.263	+.012	+.090	+.012	–.077	–	–.713	–	–	–	240	35
33	+.421[a]	–	–	–	–.219	–.305	+.123	–	–.219	–	–.403	–	–	–	360	56
35	+.579[a]	–	–	–	–.325	–1.000	–.532	–.047	–	–	–.325	–	–	–	240	59
34	+.179[a]	+.389[a]	–	–	–.164	–.088	–	–.251	–	–	–.786	–	–	–	240	50
36	+.317	+.293	–	–	–.106	–.421	–	–.242	–	–	–.421	–	–	–	240	59
37	+.506[a]	–	–	–	–.419	–.703	–1.000	–.183	–.072	–	–.183	–	–	–1.000	318	46
	–	+.007	+.147	–	–.745	–	–	–	–	+.196	–	–	–	–	160	59
38	–	(+.107)	(+.121)	–	(–.766)	–	–	–	–	(+.167)	–	–	–	–	(151)	(59)
	–	+.025	–	–	+.196	–	–	–	–.086	–	–.228	–	–	–	120	19
39	–	(+.565)	–	–	(+.085)	–	–	–	(–.190)	–	(–.326)	–	–	–	(97)	(19)
	–	–.230	–	+.249	+.185	–	–	–	–.230	–	–.230	–	–	–	100	24
40	–	(+.358)	–	(+.172)	(+.106)	–	–	–	(–.305)	–	(–.305)	–	–	–	(85)	(24)
41	–	–	+.279	–	0.000	–	–	–.126	–.503	–	–	–	+.052	–	100	45
														Total	3498	672

[a,b]See Table 4. For statistical analysis, data were combined for experiments 28 and 29, 34 and 36. Chi-square analysis was not performed on data from experiments 38–41.

TABLE 7. Numbers of prey fishes eaten by northern pike in cages in a turbid and a clearer pond in 1969. Twenty of each species of prey and two pike of 420–500 mm were put in each cage.

	No. eaten					
Secchi disc (*cm*)	Shad 67–95 mm	Carp 63–112	SMB 69–85	Shiner 94–126	Perch 83–120	
Clear 40	8	5	0	6	0	
Turbid 20	15	13	4	4	3	
		Carp 54–90 mm	SMB 62–84	Shiner 75–111	Perch 78–117	Bg 53–94
Clear 65		11	3	2	9	1
Turbid 13		8	5	4	8	0

TABLE 8. Numbers of prey fishes eaten by northern pike that had been fed golden shiner and by pike not fed golden shiner. Twenty of each species of prey and two or three pike 322–480 mm long were put in each pool.

	No. eaten				
	Buff	SMB	Shiner	Perch	Bg
Fed golden shiner	8	2	2	4	3
Not fed golden shiner	5	1	3	0	0
	Fathd	Green	Shiner	Perch	Bg
Fed golden shiner	12	6	3	2	0
Not fed golden shiner	11	8	1	2	1

the pike were larger than 165 mm. Other workers have reported that larger northern pike tend to eat larger individuals of a prey species (Frost 1954; Domaneskii 1962; Lawler 1965).

Carp longer than 110 mm were not vulnerable to northern pike up to 316 mm long (Fig. 2C, D). Similarly, Domaneskii (1962) found that carp of 125 mm and larger were not vulnerable to northern pike of 300 mm. Electivity indices for experiments in which carp larger than 110 mm long were used were corrected by subtracting the number of the larger carp from the number offered (Tables 5, 6).

Northern pike up to 446 mm long selected bluegill less than 80 mm long over larger bluegill of up to 110 mm (Fig. 2J, K). Nevertheless, electivity indices were not corrected for size of bluegill because few bluegill, including smaller, apparently vulnerable fish, were eaten. Since pike left small bluegill uneaten at the end of experiments, the presence of larger bluegill was not considered a serious source of bias.

Discussion

FACTORS AFFECTING SPECIES SELECTION

Vulnerability of prey fishes in this study may have been determined by several factors affecting preference of predator and availability of prey. Innate inclination of northern pike and appearance and behavior of prey were not controlled and may have influenced relative vulnerability. Armament probably reduced vulnerability of ictalurids, but it had no apparent effect for other species. In spite of their stout spines, carp were highly vulnerable and several species without spines were not. Distribution of prey also was not controlled, except that prey could not move far away from pike in pools, and may have affected vulnerability. Other factors were controlled to some degree, and therefore, their influence may have been minimal.

Pike had the opportunity to be satiated at all times. They could feed ad libitum in the holding pools and during experiments. Although condi-

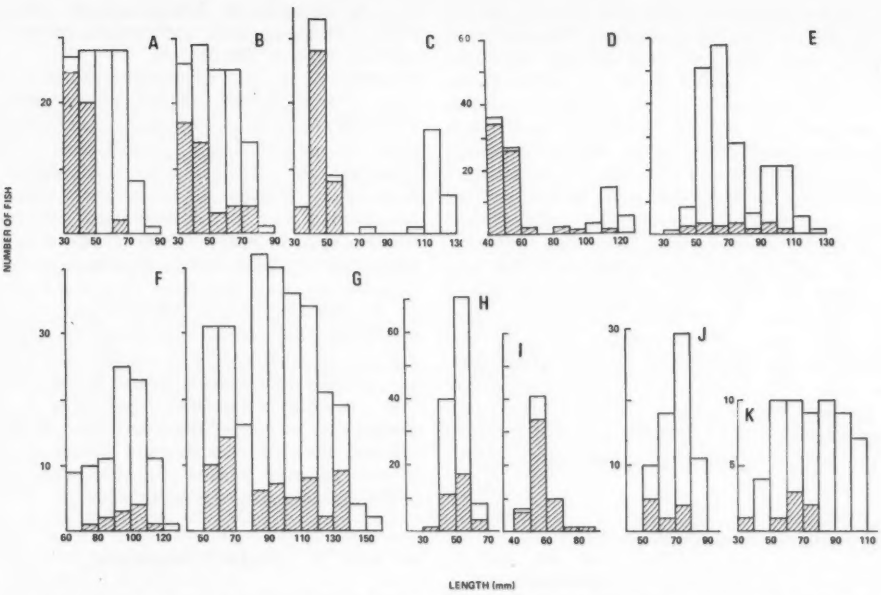

FIG. 2. Length-frequency distributions of prey fishes offered to and eaten by northern pike in pools. Total heights of bars indicate numbers offered; cross-hatching, numbers eaten. A, 120 fathead minnow, 8 pike 111–126 mm, no cover; B, 111 fathead minnow, 8 pike 147–164 mm, no cover; C, 70 carp in experiments 17, 19, 20, pike 224–303 mm, no cover; D, 60 carp in experiments 22, 23, pike 250–316 mm, cover; E, 200 golden shiner in experiments 15–18, 21, pike 224–316 mm, no cover; F, 90 golden shiner in experiments 22–24, pike 224–316 mm, cover; G, 275 golden shiner, pike 455–503 mm, no cover; H, 120 bigmouth buffalo in experiments 15, 16, 18, pike 224–316 mm, no cover; I, 60 bigmouth buffalo in experiments 23, 24, pike 224–316 mm, cover; J, 70 bluegill in experiments 19–21, pike 224–316 mm, no cover; K, 60 bluegill, pike 422–446 mm, no cover.

tioning has been observed in other species of fish (Ivlev 1961; LeBrasseur 1969), there was no evidence that conditioning influenced pike feeding in this study. Most of the pike were fed only goldfish between experiments and goldfish were not used as prey except in two experiments. Northern pike used in experiments 1–4 were fed fathead minnow between experiments and were never fed goldfish. Their selection of prey seemed to be the same as that of the other pike. Furthermore, in this study, northern pike could not be conditioned to eat golden shiner or bluegill, and Beyerle and Williams (1968) could not condition pike to eat fathead minnow, green sunfish, or pumpkinseed (*Lepomis gibbosus*).

The effect of size of prey on food selection was held to a minimum in the study, first by using prey that seemed to be vulnerable to the pike. Prey were offered that were thought to be small enough to be easily captured and swallowed by the predator but not so small as to be unattractive. Second, fishes of similar size were presented within experiments, and third, when evidence indicated that size of prey did affect the results, data were corrected to remove the effect.

Relative abundance of prey may have been of minor importance in the study. Equal numbers of prey species were offered in experiments. Relative abundance changed during experiments as fish were eaten, so that the relative abundance of preferred species declined as that of the remainder increased. The pike did not correspondingly begin rejecting those species that they had eaten first and turn to other species; if they had, all electivity values would have been zero. In experiment 1 pike ate only gizzard shad, and in experiment 2 they ate all the gizzard shad before any other species was taken. At the time a pike ate the last gizzard shad in experiment 2, there were 50 other fish present in the plastic swimming pool. Obviously something other than relative abundance determined selection of gizzard shad by pike.

Although behavior of prey may have influenced the results, no relation was obvious between vulnerability and behavior. Both gizzard shad and golden shiner schooled and were active swimmers that stayed out of cover. One was highly vulnerable to northern pike and the other was not. Carp, bigmouth buffalo, white sucker, and yellow perch schooled loosely and did not utilize cover. Fathead minnow schooled and were active swimmers that stayed near bottom along with white sucker, yellow perch, channel catfish, and black bullhead. The last two species seemed unaware of the pike and would lie under the predator as Mossman (MS 1955) observed bullheads to lie under adult largemouth bass. Green sunfish, bluegill, and largemouth and smallmouth bass occupied both open water and cover and did not school. They too showed little avoidance of pike. Attack and capture behavior of the northern pike was observed to be exactly the same as that described by Hoogland et al. (1956).

Pike Predation in Natural Waters

Results of this study may be useful in predicting northern pike feeding in natural waters. The fact that relative vulnerability was the same in ponds and pools with and without cover suggests that northern pike feeding in nature may be like that in the experiments. Northern pike appear to be selective in their feeding in natural waters (Allen 1939; Hunt 1965; data of Johnson 1969) as well as in controlled experiments such as those of this study and of Beyerle and Williams (1968). Other observations of pike feeding in natural waters that are in apparent agreement with results of this study include those of Schindler (1946) and Vaschenko (1961) who found small cyprinids and carp, respectively, to be highly vulnerable to northern pike; Frost (1954) and Lawler (1965) who reported that small pike were of negligible importance in the diet of larger pike; Allen (1939) who found no change in species eaten as pike grow larger; and F. W. Vasey (personal communication) and Kutkuhn (1958) who, with limited data, found gizzard shad to be the most important constituent of the diet of northern pike in Thomas Hill Reservoir, Missouri, and North Twin Lake, Iowa, respectively.

Perch of the genus *Perca* have been found to make up a large part of the diet of northern pike (Allen 1939; Frost 1954; Lawler 1965; Threinen et al. 1966) but were not highly vulnerable in this study. The waters studied by the first three authors apparently lacked the six species that were most vulnerable in this investigation (Fig. 1). The statement of Threinen et al. (1966) was a generalization that did not refer to specific waters; hence, it is difficult to compare it with results of this study without further details.

Northern pike may be useful in utilizing populations of nongame fishes. Gizzard shad were highly vulnerable to pike in this study and, where they occur in natural waters, gizzard shad often are abundant and distributed in both shallow and open water. Whether or not northern pike would be important predators of natural carp populations remains open to question. Small carp were highly vulnerable to pike in this study; however, there was evidence that carp may grow to a size that would be invulnerable to most northern pike within one or two growing seasons. It would seem that predictions of pike feeding in natural waters should be undertaken with consideration of the conditions that can affect vulnerability, and with an appreciation that such conditions as degree of satiation of the predator, distribution and relative abundance of prey, and sizes present of both may differ markedly from circumstances of this study.

Acknowledgments

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Life History, Ecological Production, and an Empirical Mathematical Model of the Population of *Sagitta elegans* in St. Margaret's Bay, Nova Scotia[1]

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SAMEOTO, D. D. 1971. Life history, ecological production, and an empirical mathematical model of the population of *Sagitta elegans* in St. Margaret's Bay, Nova Scotia. J. Fish. Res. Bd. Canada 28: 971–985.

The population of *Sagitta elegans* Verrill in St. Margaret's Bay, Nova Scotia, was polymodal throughout the year. The modes were distinct enough to be considered as representing subpopulations. Four subpopulations were present in the Bay during the early winter and a fifth subpopulation entered the Bay during the late winter. The growth of the subpopulations was followed through the year and related to the mean water temperature. A mathematical model describing the effect of temperature on development, length of the life cycle, and reproductive potential is presented. The model simulates the effects of temperature on the body length at maturation, ovary length, generation time, and numbers surviving at various body lengths. Predictions are made on the changes in population size due to temperature, and mortality and flushing rate changes. The calorific content of the animals at different stages of the life history are given along with the carbon content of the animals. A yearly ecological production figure is given based on the turnover rates derived from the Allen curve for one of the subpopulations. The significance of the findings is discussed in relation to the geographic distribution of the species.

SAMEOTO, D. D. 1971. Life history, ecological production, and an empirical mathematical model of the population of *Sagitta elegans* in St. Margaret's Bay, Nova Scotia. J. Fish. Res. Bd. Canada 28: 971–985.

La population de *Sagitta elegans* Verrill dans la St. Margaret's Bay, Nova Scotia, a été polymodale pendant toute l'année. Les modes étaient assez distincts les uns des autres pour permettre de différentier des sous-populations. Quatre sous-populations étaient présentes dans la baie au début de l'hiver, tandis qu'une cinquième apparaîssait à la fin de cette saison. La croissance des sous-populations a été étudiée pendant une année en fonction de la température moyenne de l'eau. Un modèle mathématique décrivant l'effet de la température sur le cycle vital et le potentiel reproductif de l'espèce est proposé. Ce modèle tient compte de l'action de la température sur la longueur des individus adultes, sur la taille de l'ovaire et la durée des générations; enfin, du rapport existant entre les survivants et la taille du corps. Des prédictions sont établies en ce qui concerne les variations de l'abondance des populations dues à la température et aux fluctuations du taux de mortalité. Les teneurs en calories des spécimens aux différents stades de leur développement sont fournies ainsi que celles en carbone. Une valeur de la production annuelle se basant sur les taux de turnover dérivés de la courbe de Allen est proposée pour une des sous-populations. La signification des résultats est discutée en fonction de la répartition géographique de l'espèce.

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A general study of the macrozooplankton of St. Margaret's Bay, Nova Scotia, was started in the fall of 1967, with the purpose of gathering information on the seasonal changes in the species composition, biomass, and production of the macrozooplankton. The most important invertebrate predator in terms of numbers and biomass at all times of the year was found to be *Sagitta elegans* Verrill. This paper describes the seasonal history and population structure of *S. elegans* during the year and presents a mathematical model that simulates changes in the population size as a result

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of changes in the mean water temperature and mortality rate using data on the survival rate, growth rate, number of eggs per unit length of ovary size at maturity, and mean water temperature during the life cycle of the animals. An estimate of the yearly production rate of *S. elegans* in the Bay is given, based on the changes in the population structure throughout the year.

In only two previous studies were attempts made to estimate the yearly production rate of *S. elegans*. McLaren (1969) attempted to do this for a population of *S. elegans* found in an Arctic lake, but was only able to sample during the summer months. Zo (MS 1969) sampled the *S. elegans* population of Bedford Basin, Nova Scotia, for 12 months and derived a production figure for the summer generation and used this value to estimate the yearly production rate.

The St. Margaret's Bay *S. elegans* population was more complex than the basin population. The basin population has two distinct generations, a spring and a fall, whereas in the bay mature spawning animals are continuously present from early spring to late fall. Therefore, the production figure given by Zo (MS 1969) could not be used for St. Margaret's Bay.

General Methods

Samples were taken from November 1968 to April 1970. All sampling was done at night, for larger numbers of animals per m² of water were caught then.

Horizontal samples were taken with a modified version of the Icelandic high-speed plankton sampler. The mouth of the sampler was enlarged to 15 cm from 7.5 cm and the surface area of net increased to give a mouth area-to-surface area ratio of 1:60. The sampler had a mesh size of 308 μ. Tows 1.5 nautical miles long (Fig. 1) were taken simultaneously at 2-, 18-, and 36-m depths at three locations inside and one outside the Bay. The path of each of the tows is shown in Fig. 1. Oblique tows were taken with a metered #6 (243 μ) mesh, $\frac{3}{4}$-m diam net and $\frac{1}{2}$-m diam net of #20 (76 μ) mesh at four stations. Samples were taken at least once a month during the winter and every 2 weeks during the summer and fall.

Samples were preserved in a 5% formalin and seawater solution, and were analyzed within 2 weeks of being collected. To determine the percent weight loss due to leaching in the preservative, six samples of live animals ranging in weight from 0.6 to 2.6 g were weighed, preserved, and weighed weekly for 3 weeks. The mean percentage losses in weight ($\pm$1 standard deviation) after successive weeks were: 8.8 $\pm$ 3.0, 12.8 $\pm$ 3.5, and 17.3 $\pm$ 5.0%.

The copepods were removed from the samples and the water filtered off under vacuum using a 30-μ mesh screen as a filter. The copepods were then weighed to the nearest 0.01 g.

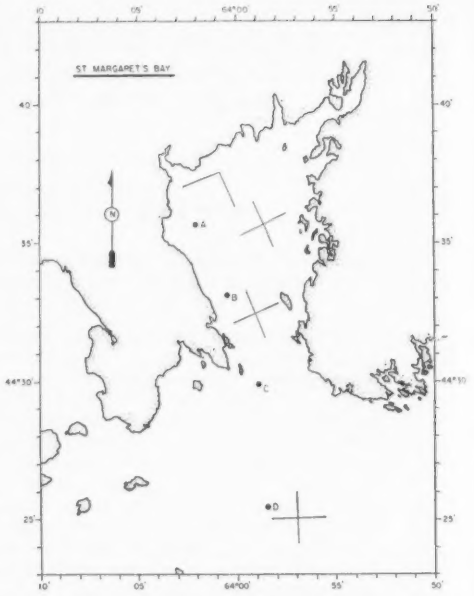


FIG. 1. St. Margaret's Bay, Nova Scotia, showing location of sampling stations. Lines indicate paths of horizontal tows, and solid circles where oblique tows were made.

Sagitta elegans in each sample were counted, and weighed after the free water was removed by blotting the animals on filter paper. They were then stained by the method described by Pierce (1941) and sorted into three stages as described by Russell (1932a). Stage I animals had faint development of the ovaries and no sign of testes; stage II had signs of testes development and well-developed ovaries without mature eggs; and stage III had large mature eggs in the ovaries. There was little doubt as to which stage the individuals were in. Spawning is believed to occur soon after the eggs mature, with the animal dying soon after it spawns. All animals produced eggs. A random portion of all the animals collected on each sampling date was selected for body length measurements. The length of the animals was measured to the nearest millimeter using the distance from the tip of the head to the base of the tail. Animals less than 5 mm were not measured, but were counted. Separate length-frequency histograms with a length class interval of 1 mm were drawn for each of the stages. The total number of animals of all stages in each size class was found, a moving average with a class interval of 2 mm calculated for these data and the smoothed frequencies plotted. An analysis was made of the polymodal distribution using the method described by Taylor (1965) and the fitted normal curves for each mode plotted. The interpretation of complex life-history data such as presented in this paper cannot help but be somewhat subjective. Taylor's method itself

is subjective to a degree, for one must decide where the peak of the first mode is and start the analysis from this point. Therefore, it was necessary to study the entire composite of modes from the different sampling dates combined with evidence from the numbers of animals in the various reproductive stages on the different dates before the reality of a particular mode was established.

The mean temperatures for the yearly temperature curve (Fig. 2) were obtained by integrating the temperatures from 0 to 50 m depth at 1-m intervals. Temperature data were collected weekly at a station near sampling station A. The mean water temperature for each subpopulation was obtained by dividing the area under the yearly mean temperature curve (Fig. 2) for the time when the subpopulation was present in the bay by the generation length of the subpopulation expressed in days. The number of degree-days between any two dates was calculated as the area under the mean temperature curve between the two dates.

Relations between length and dry weight and between live weight and dry weight were calculated by measuring live animals to the nearest millimeter and weighing them on a Cahn electrobalance to the nearest .01 mg. The animals were then dried at 60 C for 24 hr and reweighed. The dry weight represented 8.3 $\pm$ 0.2% of the live weight, based on the weights of 20 animals.

Carbon determinations were made on live animals that were oven-dried at 60 C. The analysis for percent carbon was made using the Hewlett Packard F and M 185 Carbon Hydrogen Nitrogen analyser.

The calorific values of the animals were determined using a Phillipson microbomb calorimeter. Live *S. elegans* were dried at 60 C for 24 hr and stored in a desiccator before burning.

All regression equations were calculated by the least-squares analysis method.

Identification of Subpopulations

Figure 3 gives the size distribution of each of the three stages of development and the moving average for the combined size-frequency distribution for all stages, along with the calculated normal distribution for the different subpopulations present on each of the sampling dates.

Animals less than 5 mm were not included in the histograms (Fig. 3). This does not mean that the large numbers less than 5 mm were not present in the populations. Omitting the small animals from the histograms means only that in the analyses the appearance of a new mode is not recognized until the animals are larger than 5 mm.

The relative sizes of the modes of >5 mm within each sample are taken as indices of their relative abundance. However, since different numbers of animals were measured due to random subsamples being taken from the different samples, heights of the modes between samples cannot be so employed.

The polymodal analysis on these data made it possible to identify the subpopulations. Without this mathematical treatment the interpretation of much of the data would have been very difficult, if not impossible. The fact that modes persisted in sequence from sampling date to sampling date is evidence of their reality and shows that they are not artifacts of one or two samples. The members of each mode, with few exceptions, reached the same stage of maturity at approximately the same time.

The modes detected in successive samplings occurred at larger and larger body sizes in the early stages of development. However, the rate of growth decreased as the animals approached maturity and stopped completely once stage III was reached. As a result, there is an overlapping of the modes starting with stage II, leading to an accumulation in the mature stage of groups born at different times.

Analyzing the fall samples was more difficult than analyzing the spring and summer samples due to the smaller size differences between the different stages and the shorter generation time. The relative numbers of animals in the different developmental stages on each of the different sampling dates provided the best clue to subpopulations reaching maturity.

Seasonal History and Population Structure

Four subpopulations (labelled 1 to 4 in Fig. 3) were recognized in the early winter population of 1969, with a fifth subpopulation (A) entering the bay from the coastal waters between January 10 and 23. That A was a different population of animals from that already in the bay was indicated by a sudden increase in the maximum body size of the animals in the bay between January 10 and 23 and the fact that these large animals

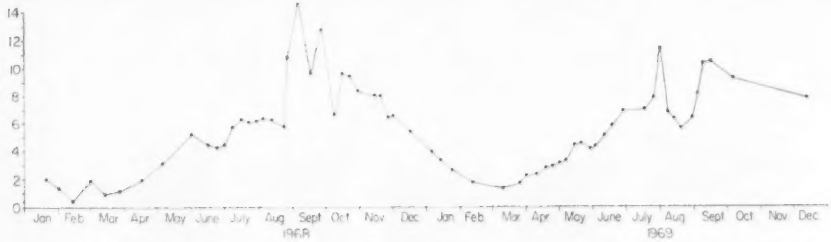


FIG. 2. Yearly mean temperature of St. Margaret's Bay at station A. Mean calculated by integrating the temperatures from 0 to 50 m at 1-m intervals.

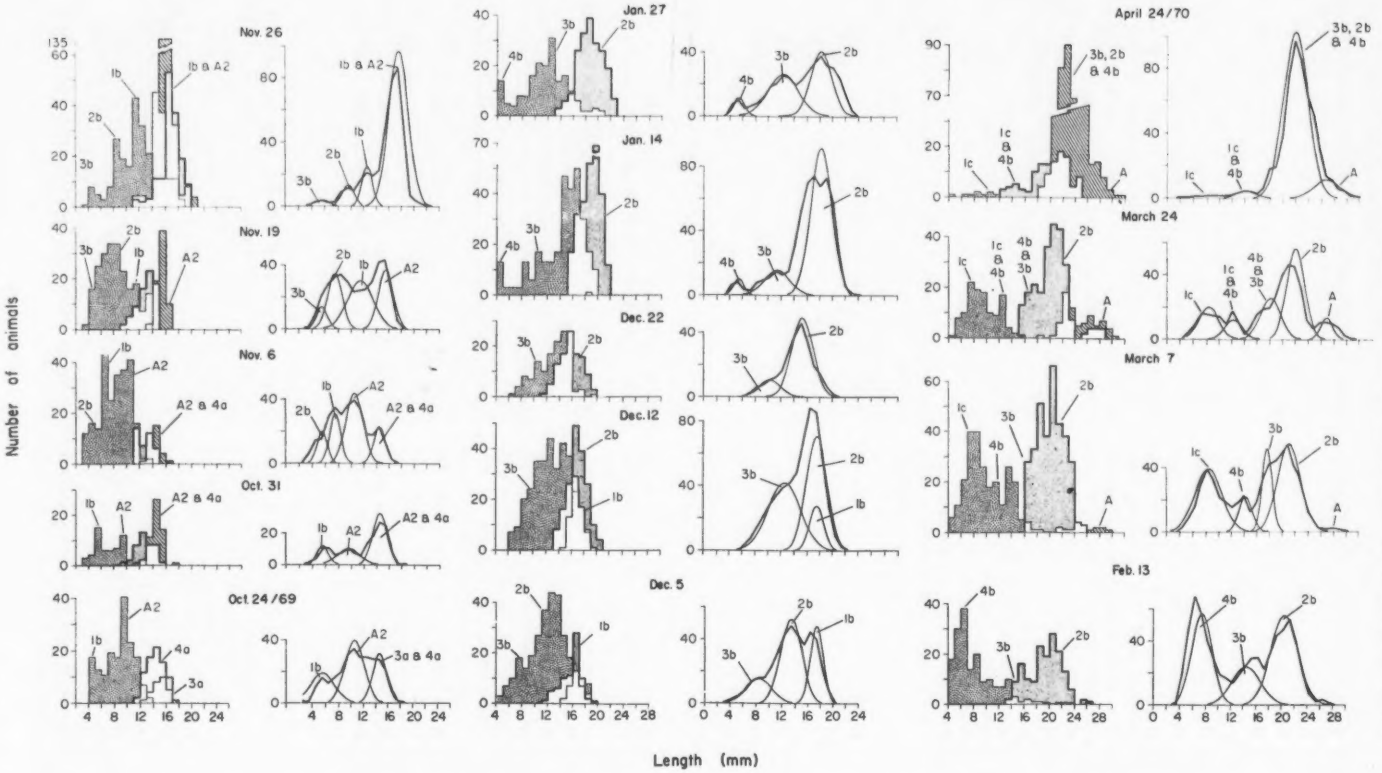
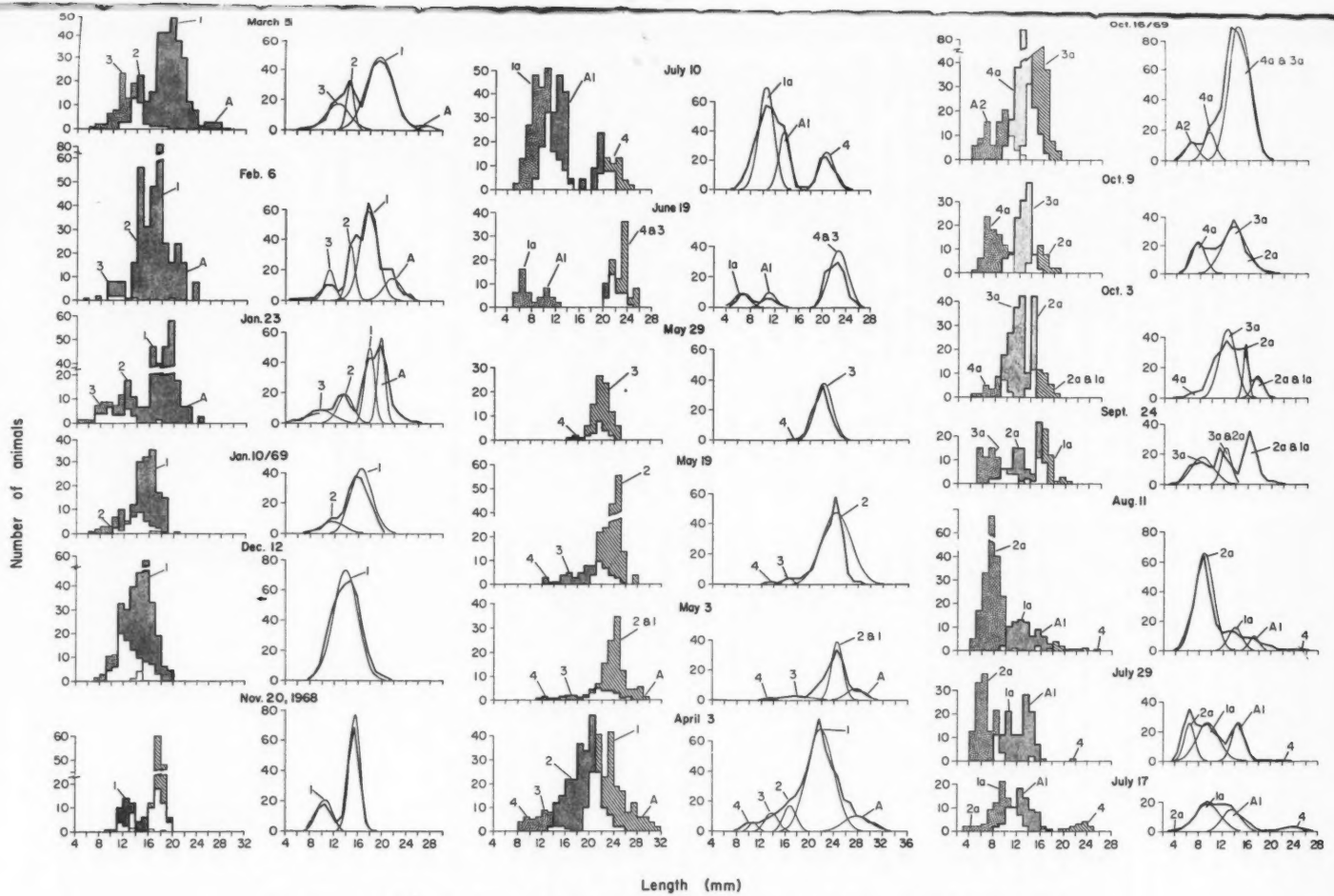


FIG. 3. Size frequency of *S. elegans* in representative random portions of all samples from the St. Margaret's Bay from November 1968 to April 1970, according to stage of development (I, stippled; II, grey; III, oblique lines); moving-average size-frequency distributions (heavy lines) for the population; and normal curves (thin lines) plotted from the polymodal analysis to identify the subpopulations (1–4, A, etc.).



Erratum : This is the first half of Figure 3, which is concluded on p. 974 (*opposite*)

were collected outside the bay before being found in it. A similar entry of large animals into the bay began about March 7 in 1970.

Spawning started in early May, for the first eggs appeared in the samples on May 3 and the maximum length of the animals in the samples decreased to 30 mm from 32 mm on April 3. The length reduction was due to breeding and then dying of the large animals (A) from outside the bay. Spawning continued into late July. It can be seen from Fig. 2 and 3 that the mean length at maturity (stage III) generally decreased during the year as the water temperature at which the subpopulations developed increased. The estimated spawning dates for each subpopulation, the mean water temperature during its life cycle, the generation length, and the degree-days to which the subpopulations were exposed are given in Table 1. It shows that the generation length of the subpopulations decreased with increased mean water temperature, but the number of degree-days each of the subpopulations was exposed to remained relatively the same.

The offspring of subpopulation A and 1, called A_1 and 1*a* appeared in the June 19 sample with two distinct modes. The large animals in stages II and III were the remnants of the overwintered population. It is inferred that the stage II animals were subpopulation 4 and the stage III animals were subpopulation 3. Subpopulation 2 spawned and died out between May 19 and May 29, during which time the mean size of the stage III animals dropped from 24.5 to 22.5 mm. The offspring of subpopulation 2, called 2*a*, appeared in the July 17 sample. Subpopulation 3 reproduced between June 19 and July 10 and the offspring, 3*a*, were represented by a prominent mode on September 24. Subpopulation 4 spawned between mid-July and early August and 4*a* appeared in the samples on October 3.

Subpopulation 1*a* reproduced and disappeared by October 9 and its offspring, 1*b*, appeared in the October 24 sample. Subpopulation 2*a* reproduced between October 9 and 16 and its offspring (2*b*) appeared in the November 6 sample. This (2*b*) was the most advanced overwintering subpopulation of the 1970 population. Subpopulation 3*a* spawned between October 16 and 31 and 3*b* appeared in the November 19 sample. Subpopulation 3*b* and 4*b* were part of the overwintering population. A_2 reached stage III by November 6 and all the members were in this stage by November 26. A_2 reproduced between November 19 and December 5. Subpopulation 1*b* matured by December 5 with some members still present in the December 12 sample, but they disappeared by December 22. Due to the lower water temperatures, slower growth rates (Fig. 5), and the close times of reproduction of subpopulation A_2 and 1*b*, their offspring blended together to form a single new subpopulation in the 1970 population, called 1*c* in the March 7, 1970, sample.

An important and complicated phenomenon occurred between December 5 and 12, 1969. On December 5 most of subpopulation 1*b* had reached the mature third stage and had ceased to grow. Subpopulation 2*b* was still in stages I and II and was still growing. By December 12, subpopulation 2*b* had overtaken 1*b* in size with the result that the size distribution of 1*b* lay within the size distribution of 2*b* (Fig. 3). Therefore, a normal curve lay within the bounds of a second normal curve. This was most obvious in the December 12 histograms, which show subpopulation 2*b* all in stage

TABLE 1. Estimated times of spawning and maturity of *Sagitta elegans* subpopulations.

Subpopulation	Birth date	Date when mature	Generation length (*days*)	Mean temp (*C*) at which subpopulation grew	Degree-days
1	Oct. 7	May 1	206	4.2	865
2	Oct. 25	May 15	200	3.8	760
3	Nov. 25	July 1	219	3.6	788
4	Dec. 15	July 15	213	3.5	745
A_1	Apr. 15	Sept. 1	142	5.2	738
1*a*	May 1	Sept. 15	137	6.1	866
2*a*	May 15	Oct. 9	147	6.8	1000
3*a*	July 1	Oct. 25	115	7.4	851
4*a*	July 15	Nov. 26	133	8.1	1077
A_2	Sept. 1	Dec. 5	95	9.0	855
1*b*	Sept. 15	Dec. 12	91	9.1	828

II completely overlapping subpopulation 1*b*, all in stage III. By December 12, 1*b* had been exposed to 828 degree-days (Table 1) and had matured and ceased to grow, whereas 2*b* had received only 540 degree-days and, therefore, had not matured and was still growing.

When reproduction resumed in 1970, subpopulation 2*b* was the first resident population to spawn, 3*b* the second, 4*b* the third, and the offspring of A_2 and 1*b* (1*c*) the last. Therefore, the resident population once more had four modes. An offshore subpopulation (A) appeared in the March 7 sample, with the result that the bay population had 5 modes, the same as in 1969.

Factors Reducing the Population

The most important factor in reducing the population of *S. elegans* in the bay appeared to be the flushing of the surface layers of water. Sharaf El Din et al. (MS 1970) found that the upper 5–10 m flushed, on the average, about once every week. Previous studies on *S. elegans* (Alvarin 1965; McLaren 1969; Zo MS 1969; Pearre MS 1970) and unpublished studies by the author, showed that young stages of *S. elegans* were found in the upper layers of water at all times of the day and night and as they matured they were found at lower levels. Therefore, it is probable that young animals were regularly removed from the Bay by flushing of the upper layers of water. The difference in age structure of the population inside and outside the bay (Table 2) supported this view. During the late winter months, there was little difference in the age structure inside and outside the bay, but during the summer and fall virtually no stage III animals were found at the outside stations C and D, but stages I and II were abundant.

It is unlikely that young *S. elegans* recruits were brought into the bay in the summer, for no significant numbers of *S. elegans* were found in two extensive zooplankton surveys of the Nova Scotia shelf during June and October 1969 (Sameoto MS 1971).

It appears that the flushing mechanism is probably the principal factor in reducing the population in the bay. Therefore, the term "survival" means the number of animals left in the bay.

Predation by young fish likely occurred during the summer and fall. These are the times when large populations of juvenile fish such as red hake (*Urophycis chuss*) and fourline snakeblenny (*Eumesogrammus praecisus*) plus a large population of mackerel and herring are present in the bay (unpublished data).

Changes in Growth Rate with Changes in Mean Water Temperature

The mean dry weights for the various subpopulations were estimated by calculating their mean lengths and estimating the corresponding mean

Table 2. Percentages of *Sagitta elegans* in samples from inside and outside St. Margaret's Bay that were in the various stages of maturity.

Date (*1969*)	Stage I		Stage II		Stage III	
	Inside	Outside	Inside	Outside	Inside	Outside
Jan. 4	1	4	76	95	23	1
Jan. 23	17		80		3	
Feb. 2	3	4	96	96	1	0
Mar. 5	24	2	74	96	2	2
Apr. 4	0	2	77	48	23	50
May 4	0	0	23	20	77	80
May 29	0		9	20	91	80
June 19	29	51	21	50	50	0
July 10	41	80	45	20	13	0
Aug. 11	25		72		3	
Sept. 15	65	99	24	1	11	0
Oct. 9	46	72	53	19	9	9
Oct. 16	18		42		40	
Nov. 6	78	80	12	20	10	0
Nov. 26	36	76	18	24	47	0
Dec. 12	34	79	48	21	18	0
Dec. 22	51	71	49	29	0	0

weights from the equation given in Fig. 4, which was obtained by regressing the body length against the dry weight of individual animals. The mean dry weights for the different sampling dates were then plotted on a log scale (Fig. 5) and linear regression equations calculated for each of the growth lines. Lines that were curvilinear were broken into segments that appeared to be linear. The growth rate, expressed in units of log dry weight, increased as the temperature (Fig. 2) increased. Growth almost stopped during February, when the mean water temperature was between 1 and 2 C, its lowest for the year. Table 3 gives the regression equations for the rate of change of log of dry body weights with time in days at the mean water temperatures calculated for the specified periods.

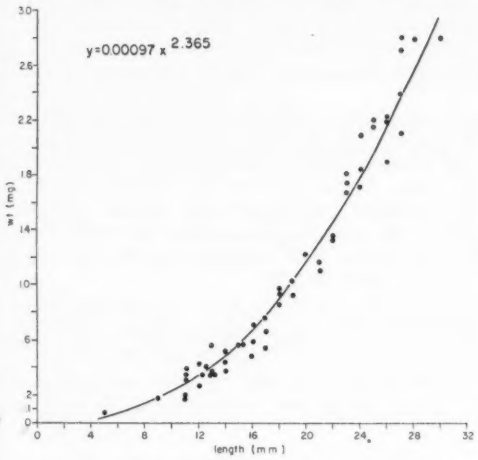


FIG. 4. Relation of weight to live body length.

A Mathematical Model of the Effects of Water Temperature on the Life History

A deterministic model was designed that plotted the survival curve related to the mean body length at maturity of each subpopulation starting with a cohort of 1000 young. The number of offspring produced in the next generation (N_l) equalled the number of animals that reached maturity in the standing population (N_o) multiplied by the mean number of eggs produced by each of the members of N_o.

It was known that the number of eggs (E) was related to the ovary length (O.L., in mm) by:

$$E = 21.6\ O.L. - 19.8 \qquad (1)$$

(McLaren 1963). The ovary length was found to be related to the body length (B.L.) at maturity (Fig. 6) by the equation:

$$O.L. = .007\ B.L.^{2.08}. \qquad (2)$$

The body length (mm) at maturity was related to the mean water temperature (T) (Fig. 7) by the equation:

$$B.L. = \frac{47.73}{T^{0.54}}. \qquad (3)$$

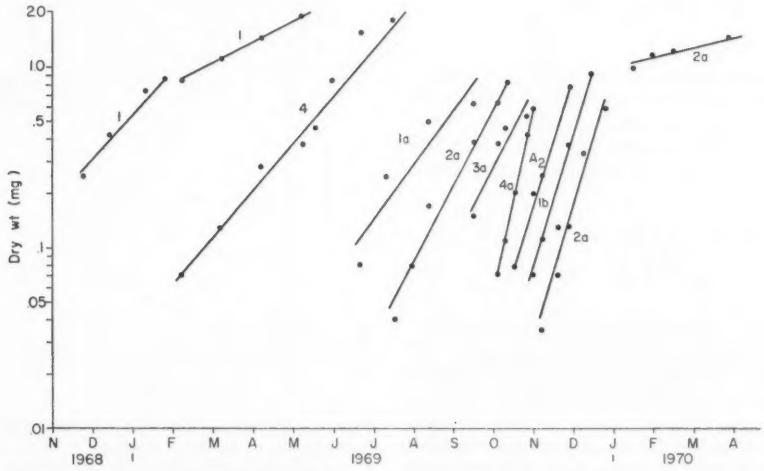


FIG. 5. Growth rates of subpopulations expressed as increases in dry body weight. Curves calculated by the least-squares method.

TABLE 3. Regression equations for growth curves of subpopulations in Fig. 3.

Subpopulation	Date	Regression equation	Mean temp (*C*)
1	Nov. 20–Jan. 23	$\log y = -.59 + .008x$	4.9
1	Feb. 6–May 3	$\log y = -.08 + .004x$	1.8
4	Feb. 6–May 3	$\log y = -1.13 + .009x$	2.0
1*a*	June 19–Sept. 15	$\log y = -0.95 + .010x$	7.4
2*a*	July 17–Oct. 9	$\log y = -1.28 + .014x$	8.3
3*a*	Sept. 15–Oct. 24	$\log y = -.77 + .015x$	9.5
4*a*	Oct. 3–30	$\log y = -1.18 + .036x$	9.2
A_2	Oct. 16–Nov. 26	$\log y = -1.09 + .024x$	8.6
1*b*	Oct. 30–Dec. 12	$\log y = -1.20 + .026x$	8.3
2*b*	Nov. 6–Dec. 22	$\log y = -1.35 + .021x$	6.9
2*b*	Jan. 10–Mar. 24	$\log y = -.02 + .002x$	<2.0[a]

[a]Estimated temperature, since no data were available.

It was found that the number of survivors (N.S.) of subpopulation 1*a* at a given time was related to the body length by

$$\text{N.S.} = \text{B.L.}^{-1.31}. \quad (4)$$

Data from the #20 and #6 net samples were used to calculate the total numbers of animals per unit volume of water for subpopulation 1*a* as it grew in length from 1 mm to maturity at 17 mm. It was possible to do this because the 1*a* mode was very distinct and, in the smaller sizes, the subpopulation overlapped only slightly with the others. The numbers of animals surviving at the various lengths were calculated on the basis of a starting cohort of 1000:

Body length (*mm*)	1	2.5	9	13	17
No. survivors	1000	293	117	29	16

Subpopulation 1*a* was the only subpopulation for which an accurate estimate could be made of

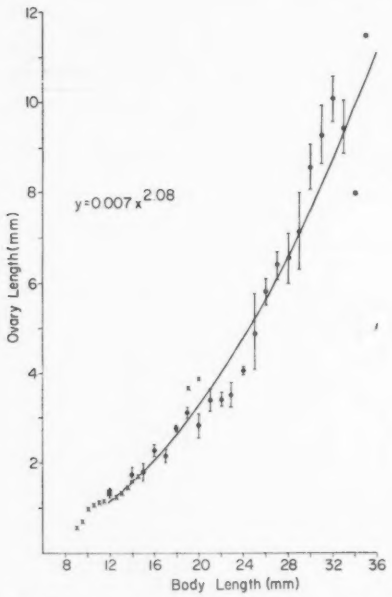


FIG. 6. Relation of ovary length to body length at maturity. ● mean length measurements ± 1 standard deviation (I). Each point is the mean of five measurements. × = values from Russell (1932a). Curve calculated by the least-squares method.

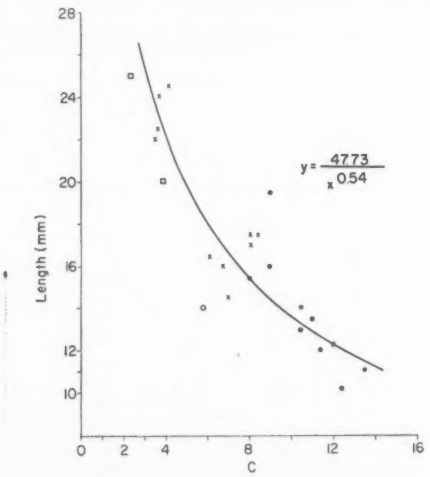


FIG. 7. Regression line for relation between mean body length at maturity and mean water temperature. × = St. Margaret's Bay; ● = Russell (1932a, b); ○ = McLaren (1969); □ = Zo (1960). Curve calculated by least-squares method.

the number of animals at the time of hatching. It was not possible to accurately estimate the initial size of the other subpopulations due to amount of overlap in the subpopulations. However, a comparison of the relative sizes of some of the other subpopulations that had distinctive modes could be made from the time they reached 5 mm. It was done by estimating the number of animals in a particular development stage, most of which was made up of the subpopulation of interest, and following the subpopulation with time and estimating its numbers per 100 m³ of water as it entered each of the three development stages. These subpopulations had similar-shaped curves to 1*a* (Fig. 8). Therefore, for the purpose of the model, it may be assumed that each subpopulation had the same survival equation as 1*a*.

The length of the life cycle in days (L.C.) was related to the mean water temperature (Fig. 9) by:

$$\text{L.C.} = \frac{611.5}{T^{0.81}}. \tag{5}$$

The number of survivors in N_t was related to the mean water temperature by substituting equation 3 for the body length in equation 4:

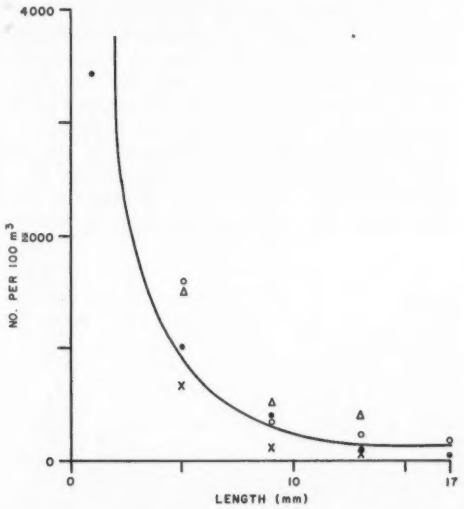


FIG. 8. Calculated survival curve for subpopulation 1*a* from hatching to maturity, and the estimated numbers of animals per 100 m³ in three other subpopulations from 5 mm to maturity. ● = 1*a*; × = 3*a*; ○ = A_2; △ = 4*a*. Curve calculated by least-squares method.

$$\text{N.S.} = \left[\frac{47.73}{T^{0.54}}\right]^{-1.31}. \tag{6}$$

The ovary length was related to the mean water temperature by substituting equation 3 for body length in equation 2

$$\text{O.L.} = .007\left[\frac{47.73}{T^{0.54}}\right]^{2.08}. \tag{7}$$

The number of eggs in the ovary was related to the mean water temperature by substituting the ovary length in equation 1 with the ovary length value in equation 7

$$E = 21.6\left(.007\left[\frac{47.73}{T^{0.54}}\right]^{2.08}\right)-19.8. \tag{8}$$

Equation 6 related the number of survivors to the mean water temperature and equation 8 related the mean water temperature to the mean number of eggs produced per individual at that temperature. Equations 6 and 8 were multiplied to give equation 9, which gave the size of the next generation (N_t) from the values of the initial size of generation N_o and the mean water temperature at which N_o grew.

$$N_t = N^o\left(\left[\frac{47.73}{T^{0.54}}\right]^{-1.31}\right) \times 21.6 \left(.007\left[\frac{47.73}{T^{0.54}}\right]^{2.08}\right)-19.8. \tag{9}$$

Time was built into the model program through the relation between size at maturity and the mean water temperature, plus the generation length and mean water temperature (Fig. 7 and 9). This made length equivalent to time

$$\text{L.C.} = \frac{611.5}{\left(\left[\frac{47.73}{\text{B.L.}}\right]^{1.85}\right)^{.81}}. \tag{10}$$

The computer plot of the model (Fig. 10) shows that the offspring of subpopulation A_2 and 1*b*, called A_3 and 1*c*, were produced close together in time, which agreed with the actual observed data.

After one breeding season the model gave a final resident population of 3787 from an initial population of 4000 residents (subpopulations 1–4) and 1000 immigrants (subpopulation A). When

subpopulation A was left out of the program the final resident population was reduced to 3265.

The relation between the biomass of *S. elegans* and that of copepods is shown in Fig. 11. In the fall of 1968 there was a much higher copepod pulse than there was in 1969, but the biomass of *S. elegans* in the fall were slightly higher in 1969. This suggested that copepods, the main food of *S. elegans*, were not a limiting factor for the *S. elegans* population in St. Margaret's Bay and using mean water temperature as the only environmental variable in the model was justified.

Effects of Temperature and Survival Rate on the Model Population

This model makes it possible to examine the effects on the population of changes in water temperature and changes in the survival rate.

An increase in the mean temperature of 1 degree C for each subpopulation reduced the final resident population to 2578 animals when starting with 4000 residents and 1000 immigrants, a reduction of 32%.

A change in the exponent of the survival equation 4 from −1.31 to −1.21 caused an increase of 26–30% in the numbers of animals in each subpopulation that reach maturity, with the subpopulations breeding at the lower temperatures having the largest increase. This increased survival of animals resulted in an increase of the final population to 7079 residents, an increase of 87%.

The mean water temperature at which the population replaced itself in the next generation depended on the survival rate. The survival equation 4 for subpopulation 1*a* gave a replacement temperature of 5.2 C. Below this temperature each succeeding generation was larger than the one before, and above it each succeeding generation was smaller. Increasing the number of animals that reached maturity by increasing the exponent of the survival equation increased the replacement temperature.

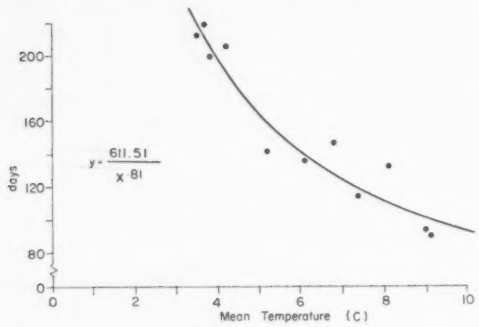


FIG. 9. Regression line for the relation between the generation length (days) and the mean water temperature. Curve calculated by least-squares method.

A Comparison of the Model and Actual Populations

The relative numbers of animals in the model population were calculated at regular time intervals and compared with the relative numbers of animals in the St. Margaret's Bay samples for the same time intervals (Fig. 12). The highs and lows for the model and the actual data agreed rather closely.

The biomass changes of the animals in the model were calculated using the equation for body length versus the live body weight (Fig. 13). This converted numbers of animals into biomass. The total biomass for all subpopulations at regular intervals of time was compared with the estimated biomass of the St. Margaret's Bay subpopulations at the same time intervals (Fig. 14). The two curves do not agree as well as those in Fig. 12, but the model does show the same general trend as the data,

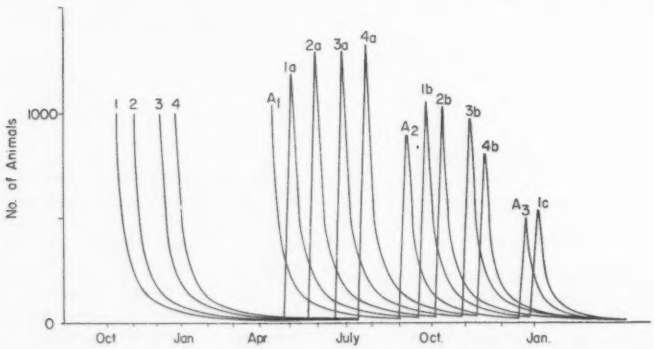


FIG. 10. Computer plot of sizes of model subpopulations with time.

with a maximum biomass occurring in the winter and a minimum in the fall.

Calorific and Carbon Content of *Sagitta elegans*

The calorific value of *S. elegans* tended to increase as the animals matured (Table 5), from about 4.0 kcal/g for first stage animals to over 5.0 kcal/g for mature third stage animals. This was likely due to the growth of gonads as the animals matured.

The carbon content of third stage *S. elegans* was 39.4 ± 2.3%, based on 10 separate determinations. This value agreed well with that of Curl (1962), who found the total carbon value for a sample of *Centropages* sp. and *S. elegans* in equal numbers to be 38.7%, and a sample of *Centropages* sp. only to be 38.5%. Therefore, *S. elegans* must have been very close to 38% carbon. McLaren (1969) gave an equation relating weight of carbon (Wc) and body length (L) of *S. elegans:*

$$Wc = 0.0356\ L^{2.93}.$$

FIG. 11. Changes with time of mean biomass of copepods and *S. elegans* in the samples expressed as g/100 m³.

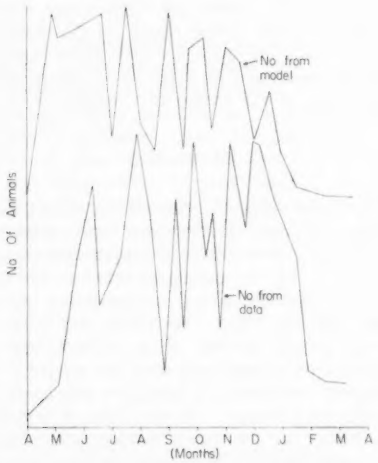


FIG. 12. Relative numbers of animals during the study period as calculated from model and as recorded from St. Margaret's Bay samples.

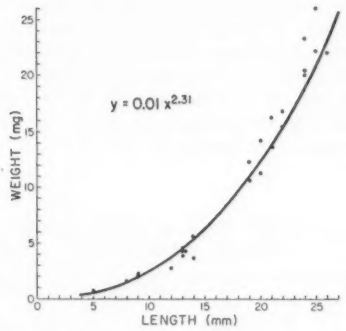


FIG. 13. Regression for relation between live weight versus body length.

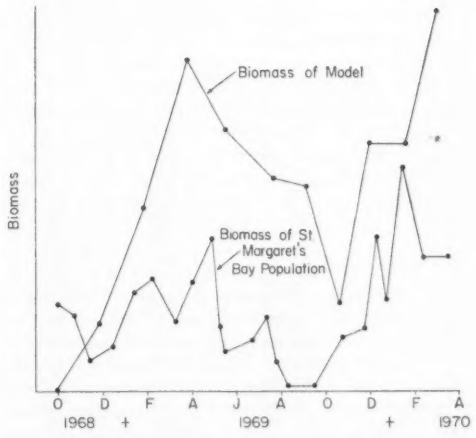


FIG. 14. Relative changes with time in biomass of the model as compared with changes of the biomass of *S. elegans* in St. Margaret's Bay samples.

When the length was converted into dry body weight by the equation in Fig. 4, and the percent carbon calculated, the values were more than 100% lower than those of this study and Curl's. Platt et al. (1969) gave an equation relating cal/g dry weight and percent carbon for mixed zooplankton:

$$\text{cal/g dry wt} = -227 + 152x\ (\%\ \text{C}).$$

When the value for percent carbon found in this study and that of Curl were put in the equation, a value of 5.7 kcal/g was obtained. This is slightly higher than the actual values found for the stage III animals (Table 4) but still is reasonably close to suggest that the percent carbon values of this study are close to the true values. The discrepancy between this value and that of McLaren's may be due to the different techniques used. McLaren used the wet-ashing method of Strickland and Parsons (1960) on fresh animals under field conditions, and it is also possible (I. A. McLaren personal communication) that errors in standardization were involved.

TABLE 4. Calorific values of *Sagitta elegans* collected from St. Margaret's Bay.

Date	Dry wt (*mg*)	kcal/g dry wt	Stage
	1968		
Dec. 30	19.93	3.999	I+II
" "	21.16	4.823	I+II
" "	11.74	4.239	I+II
	1969		
Jan. 7	17.04	5.016	II
7	9.60	4.656	II
Feb. 7	10.44	4.486	II
7	22.00	4.558	II
Mar. 24	17.44	5.216	III
	12.37	5.008	III
	12.24	4.859	III
	15.35	4.959	III
	22.94	4.551	III
	15.11	4.956	III
Apr. 10	40.86	5.125	III
	16.06	4.631	III
	21.47	4.417	III
	28.98	4.394	III
23	16.06	5.208	III
	28.98	4.599	III
	33.81	4.793	III
May 14	23.38	5.126	III
21	23.79	5.258	III

Turnover Rate and Production

An estimate of the annual turnover rate for the various subpopulations was made from the curve for the relation between the numbers of survivors and the mean body weights (Fig. 15), commonly known as the Allen (1950) curve. Data for subpopulation 1*a* were used in calculating the curve. The equation was:

$$y = \frac{0.083}{T^{0.58}}.$$

The estimated turnover rate for subpopulations 1–4 was 2.0 and for the other subpopulations 2.1. The two generations arising from subpopulation 1 (1*a* and 1*b*) each had a turnover rate of 2.1. Therefore, the yearly turnover rate of subpopulation 1 and its offspring was 6.2. The yearly turnover rate of subpopulation A and its offspring was more difficult to estimate, for nothing was known of the early life history of A, as it came from waters outside the bay. But since A gave rise to two generations A_1 and A_2, its yearly turnover rate was likely very close to that of subpopulation 1. The other subpopulations had two generations per year with a total yearly turnover rate of 4.1.

The biomass of the subpopulations continually increased even though the numbers of animals surviving decreased with time (Fig. 15). This was due to the exponential increase of body weight with increased body length (Fig. 13).

The yearly net production estimates are given in Table 5. The values per m^2/year are based on the fact that the mean depth of St. Margaret's Bay is 50 m. The value for calories/m^2 was based on the average calorific value of *S. elegans* through its life span of 4.7 kcal/g dry weight.

Discussion

This study suggests far-reaching effects of temperature on the development and productivity of *S. elegans*. These facets of temperature concerned appeared to be the mean temperature during the life cycle, and the actual temperatures prevailing during the life cycle. The lower the mean temperature during the life of the animal, the larger the animal is at maturity and the longer it takes to mature. However, at least 738 degree-days are required for maturity. The estimates of degree-days were based on the mean temperature calculated from the surface to 50 m over the life cycle of the animal. It was found that during September, when the upper layers of water were at their warmest, there were very few *S. elegans* caught in samples

TABLE 5. Production values for *Sagitta elegans* in St. Margaret's Bay.

Subpopulation	Yearly turnover rate	Biomass[a] (*g*) Mar.	Oct.	Dec.	Yearly production (*g/100 m³*)[b]
1	6.2	0.6	0.2	0.7	0.6(2.0)+ 0.2(2.1)+ 0.7(2.1)=3.1
A	6.2	0.6	0.2	0.7	0.6(2.0)+ 0.2(2.1)+ 0.7(2.1)=3.1
2	4.1	0.6	0.2	0.7	0.6(2.0)+ 0.2(2.1)=1.6
3	4.1	0.6	0.2	0.7	0.6(2.0)+ 0.2(2.1)=1.6
4	4.1	0.6	0.2	0.7	0.6(2.0)+ 0.2(2.1)=1.6
					Total = 11.0

Total yearly production of live wt 11.0 + 1.4[c] = 12.4 g/100 m³
or 6.2 g/m² per year
or 0.5 g/dry wt·m² per year
or 0.2 g C/m² per year
or 2.3 kcal/m² per year[d]

[a]Values based on assumption that each subpopulation equals one-fifth total biomass.
[b]Values based on preserved wet weights.
[c]1.4 g = weight loss in 2 weeks due to leaching in preservative.
[d]Based on mean calorific value of 4.7 kcal/g.

taken at 2 m. Therefore, using the mean temperature during the early autumn period for the degree-days calculations probably overestimated the number of degree-days the animals were actually exposed to. The values given for the winter subpopulations were likely closer to the true values, since the temperature at this time of year was relatively uniform throughout the water column.

The importance of knowing the number of degree-days needed for maturation was demonstrated when interpreting the growth of animals in subpopulations 2*b* and 1*b* in the December samples. It would have been difficult to explain why the animals in subpopulation 2*b* remained in stage II and continued to grow larger than the animals in subpopulation 1*b*, which were in the mature stage III, if it had not been known that 1*b* had received the required number of degree-days to mature and had stopped growing, whereas 2*b* had less than the number of degree-days needed for maturation.

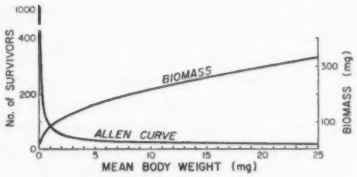


FIG. 15. Calculated Allen curve and increase in biomass for subpopulation 1*a* during the study period.

Chaetognaths, including *S. elegans* have long been used as indicator organisms for water masses (Alvarino 1965). The knowledge that at least 738 degree-days are needed for gonad maturation allows the length of the life cycle to be calculated from the mean water temperature. If the mean water temperature is not known, it can be found from its relation with the body length at maturation. These relations will make *S. elegans* more useful in identifying the water masses in which they originated.

The mathematical model was developed on the assumption that the survival rate of subpopulation 1*a* was representative of the population as a whole, an assumption that was not unjustified. Due to the complex structure of the population in the fall, it was not possible to obtain a reliable estimate of the initial population size for any of the other subpopulations. The assumption that the survival curve of the animals was the same for all subpopulations meant that the mortality rate had to increase as the growth rate increased. This was not an unreasonable possibility, for, as the water temperature increased in the bay, the number of predators of *S. elegans*, such as fish larvae and pelagic adult fish, increased. Therefore, a higher mortality rate likely occurred during the summer and fall months.

The smaller biomass of copepods in the fall of 1969 than in 1968 did not seem to affect the size of the *S. elegans* population as reflected by its biomass in the 2 years. Therefore, ignoring the availability of food in the model was justified. However, long-term studies may show a relation between the size of the copepod population and that of the *S. elegans* population.

The model demonstrated that changes in the mortality rate are more likely to influence the population size than are changes in water temperature. An increase of about 30% in the survival rate of the total population resulted in an 87% increase in the population size in the next generation. A change of 1 degree C in the yearly mean temperature caused about a 30% change in the size of the next generation. It is unlikely that a change in the yearly mean water temperature greater than 1 degree C would occur in the bay (Sharaf El Din et al. MS 1970).

The model showed that to maintain the population in the bay, it was necessary for an additional breeding stock to come into the bay from offshore waters. The model and the data demonstrated that the number of population modes did not increase each year as the offshore stock entered the bay, for, due to the closeness in time of the spawning of subpopulations A_2 and 1*c*, they blended together to form one mode. Therefore, each wintering population in the bay had four modes rather than five, as would have been the case if these two subpopulations had not come together.

Why stage III animals were not found outside the bay during the summer and fall is open to conjecture. It may be that they are swept away from the mouth of the bay and mature some place along the coast, or else, as the animals approach stage III in the water at the mouth of the bay, they move to lower depths and are swept back into the bay. If they were present at lower depths, some should have been collected, but none were found; therefore, this latter explanation is unlikely.

During the winter the population outside the bay (A) contained animals significantly longer than the population inside the bay. Animals up to 32 mm were collected outside the bay. This indicated that these animals grew in waters that were colder than those in the bay. The calculated environmental mean temperature for animals of 32 mm is 2.1 C. The relation between temperature and body length could be helpful in tracing the history of water masses, since the size at maturity is an indication of the water temperature during the animal life.

Sagitta elegans is known as an arctic to subarctic species and is found in waters with a temperature range of −0.5 to 21.0 C (Alvarino 1965). The yearly mean temperature at which the St. Margaret's Bay population replaced itself in the next generation, according to the model, was dependent on the survival rate of the parent generation. Applying the survival data for subpopulation 1*a* to the entire population gave a replacement temperature of 5.2 C. When the survival rate was held constant and the yearly mean temperature was lowered from 5.2 C, the size of the offspring generation became larger than that of the parent generation. If the yearly mean temperature was increased above 5.2 C, the size of the offspring generation was smaller than that of the parent generation.

These findings suggested that populations of *S. elegans* in regions of warmer water may be maintained in one of two ways. Large populations may be built up during the cold periods of the year, enabling the population to withstand a mortality rate higher than the birth rate during periods of warm water, or the populations may be rebuilt each year by immigration of animals from colder water.

The concept of a temperature at which *S. elegans* may replace itself in a generation was implied by McLaren (1969). He calculated a relative coefficient rate of increase (r) as a function of temperature for Russell's *S. elegans* data (1932a, b). He assumed that no mortality occurred in the population and found that $r = 0$ at about 12 or 13 C. If a mortality factor is introduced, the temperature at which $r = 0$ is lowered. This means the waters in which *S. elegans* can maintain itself without immigration must have a yearly mean temperature between 1–2 C and 12–13 C. This study showed that very little growth occurred at about 1 C. The range of *S. elegans* will be limited to waters with a mean

temperature less than 12 C due to the mortality factor. The higher the mortality rate, the colder the water must be for the population to survive.

The net productivity of *S. elegans* within the bay was 200 mg C/m², which was similar to the values given by McLaren (1969) for *S. elegans* in Ogac Lake (196–49 mg C/m² in 1957 and 318 mg C/m² in 1962). However, since McLaren's values for the carbon content of *S. elegans* are much lower than those of this study, the productivity of Ogac Lake, based on my value for the carbon content of *S. elegans* is likely higher than that of St. Margaret's Bay. The production/biomass ratios for the individual subpopulations in St. Margaret's Bay (2.0–2.1) were similar to those found by McLaren for the population in Ogac Lake. He found values ranging from 1.0 to 2.1 for different parts of the lake. Platt et al. (1969) found the net primary production in St. Margaret's Bay based on a 3-year average to be 190 g C/m²·year. This means that about 0.1% of the total yearly net primary production goes into the net *S. elegans* production.

Acknowledgments

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Trouts of the Upper Kern River Basin, California, with Reference to Systematics and Evolution of Western North American *Salmo*

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SCHRECK, C. B., AND R. J. BEHNKE. 1971. Trouts of the Upper Kern River Basin, California, with reference to systematics and evolution of western North American *Salmo*. J. Fish. Res. Bd. Canada 28: 987–998.

The five endemic species and subspecies of trout named from the upper Kern River drainage of the southern Sierras are revised to two subspecies of *Salmo aguabonita*: *S. a. aguabonita* and *S. a. gilberti*. Analysis of various characters led us to conclude that *S. a. aguabonita* did not differentiate from a coastal rainbow trout as surmised by other workers. This conclusion was based on: (1) morphological similarities with the inland cutthroat trout; (2) apparent relationships with the Gila, Apache, and Mexican golden trouts of New Mexico, Arizona, and northern Mexico, respectively; (3) a chromosomal complement identical to that of the Apache trout and more readily derived from that of *S. clarki* than from that of *S. gairdneri*; and (4) geomorphogeny and zoogeography of the southern Sierras. This evidence suggests the golden trout represents an ancient form whose origin was associated with a widely distributed group of goldenlike trouts.

The trout originally from the Kern River has a chromosome count and a chromosome arm number identical to those of *S. a. aguabonita* and therefore is classified as *S. aguabonita gilberti*. The Little Kern golden trout, *S. whitei*, is considered a synonym of *S. a. gilberti*. Similarities with an undescribed form of the McCloud-Pit drainage of northern California suggests a common ancestry. Today, *S. a. gilberti* is best represented by populations in the upper Little Kern watershed. *Salmo roosevelti* is considered a synonym of *S. a. aguabonita*, and *S. rosei* most likely had a hybrid origin derived from introductions of *S. a. gilberti* and *S. a. aguabonita*.

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FIVE forms of trouts, at times recognized as full species, have been named from the upper Kern River basin in the southern Sierra Nevada Mountains, California. The many theories and classifications proposed to explain their evolution and relationships, however, have little factual basis. The validity of taxonomic recognition of some of these forms is open to question. Primarily, however, the evolution of trouts in the Kern basin with respect to each other and to other western North American *Salmo* remains to be clarified. This paper is an attempt to do so by considering the origins, affinities, and systematic status of *Salmo aguabonita* Jordan, *S. roosevelti* Evermann, *S. gairdneri gilberti* Jordan, *S. whitei* Evermann, and *S. rosei* Jordan and McGregor. Proposed relationships are based on comparisons of Kern salmonids with cutthroat and rainbow trouts as well as two unnamed forms, one from the White Mountains in Arizona, the Apache trout, and the other from the Mount Shasta area in northern California, referred to as the redbanded trout. Judgment on the relative purity of existing populations is based on comparisons of samples collected during this study with type specimens and other museum materials.

The upper Kern River basin is defined to include the drainage of the Kern River north of Isabella Reservoir (Fig. 1). When white men first came to this area, the main Kern River, the lower Little Kern River, Golden Trout Creek, and the South Fork of the Kern River contained trouts. The only other native fish known from the entire upper basin is the Sacramento sucker, *Catostomus occidentalis* Ayres.

We do not recognize some of the scientific names used in this report; however, for descriptive convenience, to denote populations from specific localities the invalid name is used in quotation marks. For example, *S.* "*roosevelti*" is used to indicate the Golden Trout Creek stock of *S. agua-*

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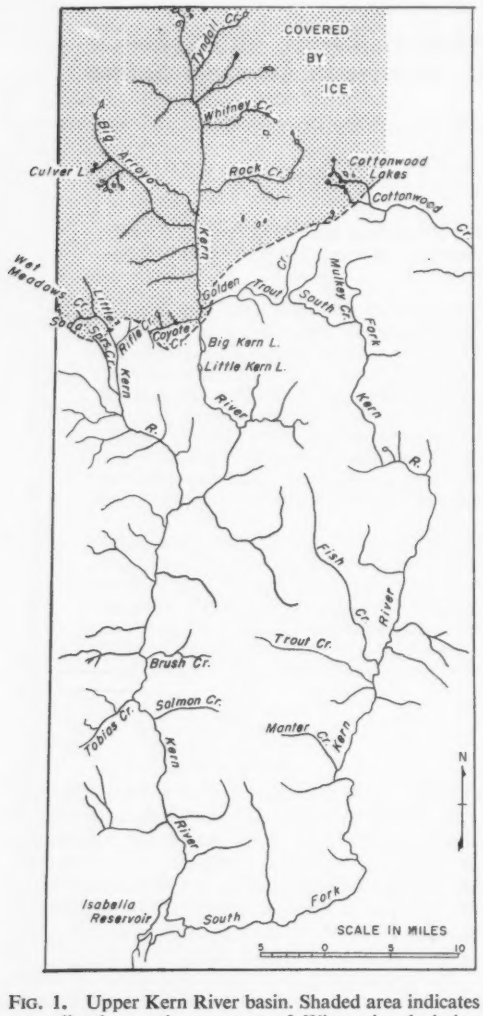

FIG. 1. Upper Kern River basin. Shaded area indicates generally the southern extent of Wisconsin glaciation in the Kern River drainage (after Matthes 1965). The extent of the ice east of the Main Crest is not indicated.

bonita. According to evidence based mainly on the karyotype of the Kern River trout, hitherto not reported, we reclassify it from *S. gairdneri gilberti* to *S. aguabonita gilberti* and use this nomenclature throughout.

This study on the Kern trouts and recent work by the junior author on other western North American trouts of dubious affinities made it apparent to us that the two endemic Kern basin trouts, *S. aguabonita aguabonita* and *S. a. gilberti*, are not local isolates of *S. gairdneri* as commonly believed. One or more major evolutionary divergences, not recently derived from either the rainbow trout or cutthroat trout series, are represented by several disjunct groups of western trouts. We conclude that both of the Kern trouts are part of a larger group of nonrainbow, noncutthroat trouts. The trouts of the Kern River basin provide important clues toward a better understanding of the origins, affinities, and zoogeography of the western trouts.

We are far from having the necessary information to make definitive conclusions and it will likely be several years before such information is available. We believe, however, that we should provide a tentative framework of theoretical evolutionary affinities, based on our evaluation and interpretation of all available information to serve as guidelines and facilitate research by future investigators. For this reason we have gone beyond the presentation of data and taxonomic revision to include speculation on phylogeny and zoogeography and suggest affinities of the Kern River trouts to other western North American *Salmo*.

Methods

Fishes were collected throughout the upper Kern River basin from 67 sites by angling in the summers of 1967 and 1968 and preserved in 10% buffered formalin. A complete list of the collections is given by Schreck (MS 1969). Coloration and spotting were observed in more than 1000 trout caught, and morphological examinations were made of approximately 500 specimens. Specimens were examined for basibranchial teeth, and principal rays of dorsal, anal, pectoral, and pelvic fins and of branchiostegal rays were counted. Vertebral numbers were obtained by X-ray and include every ossified centrum. Scales in the lateral series were counted (Hubbs and Lagler 1947) in rows running obliquely to the lateral line from the anterior-most scale two rows above the lateral line, to the scale at the distal end of the hypural complex, the site of which was determined by flexing the caudal peduncle. Pyloric caeca counts were made by considering each complete tip as a single element. Gillrakers, including rudiments, were counted on the first left arch.

Excepting museum specimens, the specimens collected (recorded as 1967 or 1968) and those furnished by D. P. Christenson, California Department of Fish and Game (collected in 1968), are in the collections of the Colorado Cooperative Fishery Unit, Colorado State University. Depositories, identification numbers, and collection dates of museum samples are as follows:

S. aguabonita gilberti: Stanford University, S.U. 11509, 4841, 4747, July 1893; S.U. 13017, 13103, July 1904. Academy of Natural Science of Philadelphia, P.A.N.S. 31785, 31786, July 1904.

S. "rosei": Stanford University, S.U. 16717, Sept. 15, 1923.

S. "*whitei*": Stanford University, S.U. 9245, 9831, July 1904. California Academy of Sciences, Indiana Museum numbers 1112 and 2866 (0900). July 1904.

S. aguabonita: California Academy of Sciences, Indiana Museum number 1117, 7-04, tag 2998, July 26, 1912.

S. "*roosevelti*": Stanford University, S.U. 9255, 4842, July 1904.

Historical Background and Critique

Kern River Trout

Jordan and Henshaw (1878) first discussed the trout of the Kern River and referred to it as both *S. irideus* Gibbons and *S. tsuppitch* Richardson. Jordan (1894a) described *S. gairdneri gilberti* from the main Kern River. Jordan (1905, 1906, 1915) and Evermann and Clark (1931) incorrectly listed the range of *S.* "*g. gilberti*" to include the Kings, Merced, and Tuolumne rivers. Theories concerning the origin of *S. a. gilberti* are: (1) that it represents a transitional stage in the evolution of the rainbow trout from a cutthroat trout progenitor that migrated from the Colorado River basin into the Kern area (Jordan 1894a, b); or (2) that it differentiated from a local population of *S. gairdneri* isolated in the Kern River and that it was the parental form of the other forms of Kern trouts. This latter view has been generally accepted. Jordan (1928), however, altered his opinion and stated the Kern golden trouts (*S. aguabonita*, *S.* "*roosevelti*," and *S.* "*whitei*") were derived from the Shasta rainbow trout, *S.* "*shasta*" Jordan. Most recent workers consider the trout of the Kern River as a subspecies of *S. gairdneri* (Shapovalov et al. 1959).

Little Kern Golden Trout

Evermann (1906) described *S. whitei* from the South Fork of the Kaweah River where it had been introduced from Soda Springs Creek, a tributary to the Little Kern River. He believed that this goldenlike trout was endemic to the Little Kern drainage and to Coyote Creek, a tributary to the main Kern River. Dill (MS 1945) stated that *S.* "*whitei*" may not have originally extended throughout the upper Little Kern drainage, and Ellis (MS 1915) listed a stocking record for the upper Little Kern River with fish taken in 1879 from the lower river, below the falls at Soda Springs Creek. A most significant point, not known to Evermann when he described *S.* "*whitei*," is that all the populations of *S.* "*whitei*" collected by him were derived from introductions originally from the lower Little Kern River below the falls at the junction of Soda Springs Creek.

The trout of the Little Kern River basin has been classified as a species, *S. whitei*, or as a subspecies of *S. aguabonita*. It is generally assumed by the subspecies classification that *S. a.* "*whitei*" and *S. a. aguabonita* differentiated independently from *S. a. gilberti*. Such reasoning implies that *S. aguabonita* is a polyphyletic species.

Trout from Culver Lake

Jordan and McGregor (1924) named the trout they considered native to Culver Lake (largest of the Little Five lakes) and adjoining lakes *S. rosei*. They believed it was descended from a *S. a. gilberti* and would ultimately be classified as a subspecies of it. Dill and Shapovalov (1954) stated that *S.* "*rosei*" is a synonym of *S. a. gilberti* because Culver Lake, originally barren, was planted with fish from the Big Arroyo; they assumed *S. a. gilberti* was indigenous to this stream. Natural barriers on the Big Arroyo allow little possibility that fish were native to this stream. Evermann (1906) listed an introduction (1894) into the Big Arroyo of fish from the Little Kern River but later (Evermann 1909) acknowledged a report that it was stocked with Kern River fish. Ellis (MS 1915) reported that this introduction was in the 1870's. Jordan and McGregor (1924) listed an introduction of trout from Golden Trout Creek (*S.* "*roosevelti*") into the Big Arroyo but gave no date of this stocking. Thus the type specimens of *S.* "*rosei*" may be hybrids between *S. a. aguabonita* and *S. a. gilberti*.

Golden Trout of the South Fork of the Kern

The trout of the South Fork of the Kern River, because of its striking similarity in spotting pattern and coloration to the interior cutthroat trout, was referred to as *S. pleuriticus* Cope by Jordan and Henshaw (1878). Jordan (1892) described it as a subspecies of cutthroat trout, *S. mykiss aguabonita* (written *Salmo mykiss agua bonita* in the title of the original description and *S. m. augabonita* in subsequent papers by Jordan), from specimens he thought were from Golden Trout Creek. The description of *S. aguabonita* as a subspecies of *S. mykiss* Walbaum was based on an erroneous assumption that the cutthroat trout species distribution extended across the North Pacific to Kamchatka. *Salmo mykiss* of Kamchatka is not a cutthroat trout but is closely related to and probably conspecific with *S. gairdneri* (Behnke 1966). Evermann (1906) corrected the type locality to Cottonwood Creek, where *S. aguabonita* was transplanted in 1876 from Mulkey Creek, a tributary of the South Fork of the Kern River. Jordan (1893) republished the description of *S. aguabonita* and

stated that it was an offshoot or descendent of the cutthroat trout found between the Sierra Nevada and the Rocky Mountains. Jordan omitted the remark that *S. aguabonita* "was in no way related to the rainbow" made in the 1892 description. Subsequently, however, he considered the golden trout to be a subspecies of *S. gairdneri* (Jordan 1894b). Currently, it is recognized in most literature as *S. aguabonita*, and most authors have accepted the assumption that it differentiated from an isolated population of trout from the Kern River. Ferguson (1916) believed that the presence of the golden trout in the Kern area antedates that of the Kern River trout. Needham and Gard (1964) suggested that *S. a. aguabonita* may have close affinities with the Mexican golden trout, *S. chrysogaster* Needham and Gard, of Mexico, and the Gila trout, *S. gilae* Miller, of New Mexico. Behnke (1970) discussed the affinities of *S. a. aguabonita* with those of the Apache trout of the White Mountains in Arizona.

Golden Trout of Golden Trout Creek

Evermann (1906) named the trout of Golden Trout Creek *S. roosevelti* and separated it from *S. aguabonita*, due to differences that he stated existed in the distribution of spots and in coloration. According to Evermann, the spots of *S.* "*roosevelti*" are restricted posterior to the dorsal fin, whereas those of *S. aguabonita* extend more anteriorly. Jordan (1911) wrote that he was "unable to see how the species of Evermann, *Salmo Roosevelti*, differs from the original, *Salmo aguabonita*."

Curtis (MS 1934a, 1934b, 1935) stated that scale counts and spots of *S.* "*roosevelti*" were not significantly different from those of *S. aguabonita* and concluded that "*Salmo roosevelti* is not a distinct species, but a color variant of *Salmo agua-bonita*." Since then, *S.* "*roosevelti*" has not been recognized as a distinct taxonomic entity, but Miller (1950) wrote "...the golden trout may well represent only 1 or 2 species..., but critical studies of these forms in their native habitat are yet to be made in order to clarify their status."

Antigenic differences demonstrated by Calaprice and Cushing (1964, 1967) between stocks of Golden Trout Creek and the South Fork of the Kern are open to interpretation. Isolated populations of the same species have different gene frequencies determining blood proteins. Consequently, this technique has limited value in assessing the magnitude of genetic divergence for taxonomic classification.

Despite the voluminous literature, the fact remains that no adequate description or diagnostic characters of the trouts from the upper Kern River basin have been published.

Critique

Needham and Gard (1959) showed that the trouts of the Kern River basin freely hybridize with each other and with rainbow and cutthroat trouts. Stocking records tabulated and summarized by Schreck (MS 1969) reveal that Golden Trout Creek and the upper South Fork of the Kern probably never received introductions and that these two streams served as the major source of golden trout introduced into new waters. Intentional mixing of formerly isolated native populations and widespread indiscriminate introductions of exotic trouts have greatly endangered the genetic integrity of indigenous forms and confounded taxonomic investigation.

Morphometric characters are not diagnostic for the Kern trouts, and data presented by Needham and Gard (1959) generally demonstrate that such criteria have little discriminatory value in North American *Salmo* taxonomy.

Meristic characters shown in Table 1 were found to contain the most taxonomic information about Kern trouts. Data on key characters from important samples are presented in Table 2. The fact that meristic characters may be modified by the environment (Barlow 1961; Garside 1966; Tåning 1952; Vladykov 1934) is appreciated, but many authors place undue emphasis on variability induced by laboratory experiments. From unpublished data from thousands of specimens of the rainbow and cutthroat trout groups representing localities from Alaska to Mexico and from diverse habitats, we conclude that certain meristic characters are relatively stable and validly reflect the genotype. Characters presented in Table 1 as "typical" for *S. gairdneri*, *S. clarki*, and the Apache trout are based on these data. Meristic characters most useful for distinguishing certain species and subspecies of western North American *Salmo* are the numbers of vertebra, scales in the lateral series, scales above the lateral line, pyloric caeca, and pelvic fin rays. We have data (Schreck and Behnke unpublished data) comparing samples of introduced populations with the parent stocks of *S. a. aguabonita*, *S. a. gilberti*, *S. clarki henshawi* Gill and Jordan (Heenan Lake stock), and *S. c. lewisi* (Girard) (Yellowstone Lake stock) demonstrating the relative stability of meristic count when similar genotypes are reared in different environments. Scale counts exhibit the most nongenetic variability (up to ±5% change in mean values). The number of vertebra, pyloric caeca, and fin rays appear little changed under natural conditions in different waters (typically no more than 2% variation in mean values). Low numbers of vertebra and pyloric caeca are characteristic of *S. a. aguabonita*. High scale counts

TABLE 1. Characters typical of cutthroat, rainbow, Apache, golden, and Kern River trouts (see text for explanation of how these values were obtained). A = acrocentrics; M = metacentrics.

Character	*S. clarki*, cutthroat trout		*S. gairdneri*, rainbow trout		Apache trout		*S. a. aguabonita*, golden trout		*S. a. gilberti*, Kern River trout	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Vertebrae	62	60–64	63.5	61–65	59.5	58–61	59.4	58–61	61	60–63
Scales in lateral series	170	140–200	133	110–150	155	138–168	175	150–210	154	140–170
Scales above lateral line	40	32–48	28.5	25–32	36	33–40	39.5	34–45	36	28–42
Gill rakers	19[a]	15–23	19	16–22	19.5	18–21	19	17–21	19.8	17–22
Pyloric caeca	40[a]	30–50	50	35–70	30	22–40	30.5	25–40	36(?)	30–50(?)
Pelvic rays	9	8–10	10	9–11	9.5	9–10	9.1	8–10	9.7	9–11
Karyotype	2N (A) (M) Arms		2N (A) (M) Arms		2N (A) (M) Arms		2N (A) (M) Arms		2N (A) (M) Arms	
	S. c. clarki									
	70[b] 34 36 106		60[b] 16 44 104		58[c] 10 48 106		58[b] 10 48 106		58[d] 10 48 106	
	S. c. lewisi									
	64[b] 22 42 106									
	S. c. henshawi									
	64[b] 22 42 106									

[a]Excepting *S. c. henshawi*, which has more.
[b]Simon MS 1964.
[c]Behnke 1970.
[d]These counts were made in July 1970, by R. Wilmot and R. C. Simon, Oregon Cooperative Fishery Unit, Oregon State University, from specimens obtained from upper Soda Springs Creek by the California Department of Fish and Game at our suggestion.

TABLE 2. Frequency distributions of meristic characters for Kern trout samples.

Form and date(s) of collection	Vertebra							
	58	59	60	61	62	63	64	Mean
S. "roosevelti"								
Golden Trout Cr., types and others, 1891, 1904, 1956, 1967, 1968	5	40	13	4				59.26
S. a. aguabonita								
S Fork of the Kern, Mulkey Cr., Cottonwood Cr., 1892, 1912, 1956, 1968	13	44	40	10	1			59.46
S. "whitei"								
Little Kern R., etc., coll. by Evermann, 1904			2	2	2	2		61.50
Soda Springs Cr., 1968		1	2	8	1			60.75
Wet Meadows Cr., 1968			3	2	7	1		61.46
Rifle Cr., 1956, 1967			6	12	8			61.08
S. a. gilberti								
Kern R., including types, 1893			2	8	2			61.00
Kern R., coll. by Evermann, 1904				3			1	61.75
Hybrids								
Kern R. at Soda Springs, 1968				7	8	5	2	62.08
Big Kern L., 1968			2	4	7	2		61.60
Culver L., *S. "rosei"* types, 1923			1	1	1			61.00
Salmon Cr., 1961, 1967		6	4	10	3	2		60.64

(*Continued*)

TABLE 2. Frequency distributions of meristic characters for Kern trout samples. (*Concluded*)

	Scales in lateral series										
	120–129	130–139	140–149	150–159	160–169	170–179	180–189	190–199	200–209	210–219	Mean
S. "roosevelti"											
Golden Trout Cr., types and others, 1891, 1904, 1956, 1967, 1968			1	5	12	9	10	3		1	172.8
S. a. aguabonita											
S Fork of the Kern, Mulkey Cr., Cottonwood Cr., 1892 1912, 1956, 1968				2	2	10	6	5	1	3	183.4
S. "whitei"											
Little Kern R., etc., coll. by Evermann, 1904			1	2	5						159.0
Soda Springs Cr., 1968			2	4	1						152.0
Wet Meadows Cr., 1968		2	7	6	1						148.3
Rifle Cr., 1956, 1967			7	4	4	3	1				155.3
S. a. gilberti											
Kern R., including types, 1893			3	2		1					152.6
Kern R., coll. by Evermann, 1904		1		3							152.7
Hybrids											
Kern R. at Soda Springs, 1968	2	4	13	7	2						144.5
Big Kern L., 1968	1	2	9	3							144.0
Culver L., *S. "rosei"* types, 1923 (no. approximate)				1	1	1					162.3
Salmon Cr., 1961, 1967	1	4	6	8	5	1					150.0

	Pyloric caeca									
	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	Mean
S. "roosevelti"										
Golden Trout Cr., types and others, 1891, 1904, 1956, 1967, 1968		11	10	6						32.2
S. a. aguabonita										
S Fork of the Kern, Mulkey Cr., Cottonwood Cr., 1892, 1912, 1956, 1968		10	6	1						29.7
S. "whitei"										
Soda Springs Cr., 1968		2	6	4	1					34.0
Wet Meadows Cr., 1968		1	3	5	3	1				37.5
Rifle Cr., 1956, 1967		2	6	9	1	1				36.6
S. a. gilberti										
Kern R., coll. by Evermann, 1904				1	1					40.0
Hybrids										
Kern R. at Soda Springs, 1968		1	1	7	3	3	5	1	2	45.5
Big Kern L., 1968				3	3	2	2		1	45.7
Salmon Cr., 1961, 1967	1	10	7	5	1					31.5

generally are indicative of the former and cutthroat trouts. Low numbers of scales and high numbers of pyloric caeca and vertebra are typical of *S. gairdneri*. High gill-raker counts are useful to distinguish *S. c. henshawi* from other western North American trouts. Cutthroatlike trouts typically have nine pelvic fin rays whereas rainbow trouts generally have 10.

Coloration received considerable attention by describers of the Kern trouts. Jordan (1905) reported that *S. a. aguabonita* "will possibly be found to change back to the original type if propagated in different waters." This assumption may have been based on the premise, repeated by Evans et al. (1955) that the coloration of *S. a. aguabonita* is due to the mineral content of the ambient water and to substrate brilliance. The Fresno Republican (1912) printed a statement by A. D. Ferguson that this popular belief is in error and that coloration is a "fixed character." Constancy of coloration of *S. a. aguabonita* raised in lakes and hatcheries was cited as proof. Needham and Gard (1959) reported that *S. a. aguabonita* brought to Hot Creek State Fish Hatchery, California, and Steinhart Aquarium, San Francisco, lost their bright colors. This can be attributed to a genetotrophic artifact as discussed by Williams (1963). The crux of the matter is not the ability to lose or change coloration, but the presence of a genotype that allows development of a particular pigmentation. The genetic basis of *S. a. aguabonita* markings and coloration can be appreciated from comparisons of this form from different localities and habitats.

The color plate of *S. "roosevelti"* published by Evermann (1906) is an accurate representation of the color pattern of a "typical" *S. a. aguabonita*. Salient color markings of *S. a. aguabonita* are: sides gold to yellow, lateral band red, venter gold-red, cutthroat marks yellow or faint, but the entire ventral region of the head often crimson. The dorsal fin has an orange tip, and the pelvic and anal fins have a white leading border or tip. Spots of *S. a. aguabonita* are large and are mainly restricted to the posterior region of the body, a pattern virtually identical to that of most subspecies of interior cutthroat trouts. Spots may, however, extend anteriorly above the lateral line from the dorsal fin to, or onto, the head. Despite Evermann's opinion, there are no consistent differences in spot distribution between *S. "roosevelti"* and *S. a. aguabonita*.

The color plate of *S. "whitei"* in Evermann (1906) is actually the most accurate representation of the native trout from the Kern River, *S. a. gilberti*. Distinguishing colorations are the yellow cutthroat mark and brick-red lateral band. The dorsal fin with a yellow-orange tip and anal and pelvic fins with white leading margins or tips, or both, are distinctly marked as illustrated in Evermann's report. Spotting of *S. a. gilberti* is intermediate between that of *S. a. aguabonita* and *S. gairdneri*.

Dentition on the basibranchial plate is a primitive salmonine character present in cutthroat trouts and always lacking in the rainbow trout. An important finding of this study is that no basibranchial teeth were found in *S. a. aguabonita* but were present in some of the original specimens of *S. a. gilberti*. One of six trout collected in the Kern River in 1893 (Stanford Univ. 4747) had two basibranchial teeth. Of four examined Kern River specimens collected in 1904, two (Phila. Acad. 31785 and 31786) had 16 and 17 teeth respectively. Two of 28 fish from the Kern River and 4 of 15 trout from Big Kern Lake collected in 1968 had these teeth. It is significant that basibranchial teeth were present in the Kern River population prior to the stocking of cutthroat trout in 1895 and 1896 cited in Anonymous (1896).

Systematic Status of the Trouts

Comparison of all characters examined, and notably the frequency distributions of characters shown in Table 2, indicates that *S. "roosevelti"* and *S. a. aguabonita* are similar, and the former should be considered a synonym of the latter. Slight differences observable between stocks from Golden Trout Creek and the South Fork of the Kern in our opinion do not warrant formal taxonomic recognition. Table 3 summarizes our revised classification of all Kern River basin trouts.

Hubbs (1943) discussed criteria for genera, species, and subspecies in fishes emphasizing the practical criterion of degree of differentiation. The widely accepted "biological species" concept of Mayr et al. (1953), emphasizing reproductive isolation, has limitations when applied to some groups of freshwater fishes. There is no known genetic incompatability between any group of western North American *Salmo*. Coastal rainbow and coastal cutthroat trouts do occur sympatrically and rarely hybridize. This provides the basis for recognition of *S. gairdneri* and *S. clarki* as valid species and conforms to Mayr's (1969) criteria for this taxon.

Detailed information on fertility of *S. aguabonita* × *S. gairdneri* and *S. aguabonita* × *S. clarki* hybrids is lacking. Gould (1966) reported the results of fertilizing *S. a. aguabonita* eggs with *S. clarki* sperm. It was estimated that more than 90% of the eggs hatched, but severe mortality occurred in the advanced fry stage. It should be pointed out that interspecific crosses made with the aim of determining genetic compatibility and evolutionary affinities of the parent species must involve complete four-way matings if any valid interpretation is to be made from negative results. Unless the eggs and sperm of both sets of male and female parents of the two species involved, along with the

TABLE 3. Revised classification and synonomy of Kern River basin trouts. See text for basis of revisions and Fig. 1 for localities.

Original name, type locality, and common name	Revised classification	Native range	Current distribution
Salmo mykiss agua bonita Jordan (1892): Cottonwood Cr., based on introduction from S Fork of Kern basin; S Fork of Kern golden trout *Salmo roosevelti* Evermann (1906): Golden Trout Cr; golden trout of Golden Trout Cr.	*S. aguabonita aguabonita*	Golden Trout Cr., and S Fork of the Kern R.	Golden Trout Cr., S Fork of the Kern R., many waters tributary to the Kern R. from the east and now widely distributed in western U.S.
Salmo gairdneri gilberti Jordan (1894): Kern R. at Soda Springs; Kern R. trout *Salmo whitei* Evermann (1906): Upper Little Kern R. basin (type from S. Fork of the Kaweah R. where it had been stocked from the Upper Little Kern R. basin; Little Kern golden trout	*S. aguabonita gilberti*	Main Kern R. and Lower Little Kern R.	Restricted to the upper Little Kern R. basin
Salmo rosei Jordan and McGregor (1924): Culver L.; rose trout	Most likely a hybrid based on introductions and not a valid taxonomic entity		

two hybrid lots produced by reciprocal matings, are reared under identical conditions, the nongenetic effects of degree of ripeness and overall quality of the eggs and sperm cannot be separated from the effects of genetic compatability determining the viability of hybrids.

It is evident from field observations that *S. aguabonita* readily hybridize with both *S. gairdneri* and *S. clarki* and the hybrids must be relatively fertile. Rainbow × golden trout hybrids were commonly encountered in many waters of the Kern basin during this study, and we know of no example where the forms have maintained genetic integrity if both were present in waters with spawning areas. Examination of specimens collected in 1969 and 1970 revealed that an introduction of cutthroat trout into the Alpine Lake watershed, Wind River drainage, Wyoming, is producing a hybrid population.

The junior author, using a comparison of standard taxonomic characters, examined 11 specimens collected from Alpine Lake in 1969, and Dr R. Simon, Oregon Cooperative Fishery Unit, compared karyotypes of 10 specimens collected from Alpine Lake in 1970. Simon and Behnke independently concluded that one specimen in each sample represented the offspring of a hybrid backcrossed to *S. aguabonita* (Simon personal communication).

The outlet stream of Roxy Ann Lake in the headwaters of the North Platte River system, Jackson County, Colorado, contains a trout possessing a thorough intergradation of characters representative of *S. aguabonita*, *S. gairdneri*, and *S. clarki*, which have been stocked in the lake. This mixture of the three species could not occur if the hybrids were not viable. Because of this lack of reproductive isolation, *S. a. aguabonita* does not conform to the category of a "biological species." *Salmo gairdneri* and *S. clarki* are recognized as valid species, but to relegate *S. aguabonita* to a subspecies of either of these taxa would be entirely arbitrary. Zoogeographic and taxonomic evidence suggests that *S. a. aguabonita* is part of a distinct evolutionary group referred to here as the golden trout complex. Therefore, based on current knowledge of origins and affinities, the California golden trout is best recognized as a full species, *S. aguabonita*.

Meristic characters listed in Table 1 show *S. a. aguabonita* more similar to *S. clarki*-like trouts than to *S. gairdneri*. Coloration of *S. a. aguabonita*, particularly reds and yellows, are also more similar to that of cutthroat trout. Spots of typical inland cutthroat trout are larger, more nearly circular, and more pronounced than those of rainbow trout and are predominately concentrated posterior to the dorsal fin. The spotting pattern of *S. a. aguabonita* is very similar to those of many interior cutthroat trout.

Comparison of karyotypes given in Table 1 shows that the chromosomal complements of *S. a. aguabonita* and *S. a. gilberti* are similar to the Apache trout's with respect to number and arm ratios. Centric fusion (Robertson's Law), discussed by Simon (MS 1964) and Simon and Dollar (1963), can be applied to derive this karyotype more readily from an *S. clarki* ancestor than from *S. gairdneri*.

Salmo aguabonita gilberti is now best represented by certain populations in the upper Little Kern drainage. This conclusion is based on comparison of specimens of trout from the Kern River collected prior to 1900, including the type series, those obtained by Evermann in 1904 from the Kern River and Little Kern basin, and trouts in our collections (Table 2, and data presented in Schreck (MS 1969)). This finding is also based on a comparison of the illustration of *S. a. gilberti* in the original description by Jordan (1894a) and those made by Evermann (1906). Until now, Evermann's color plates served as the basis of recognition for *S. a. gilberti* and *S. "whitei,"* because no comprehensive data have been published.

It is evident from the ranges and means of the characters listed in Table 2 that the trout named from the Kern River in 1894 has no apparent differences from the trout described as *S. whitei* from the Little Kern basin in 1906. The spotting patterns of Jordan's Kern River trout and Evermann's *S. "whitei"* are similar. Jordan described *S. a. gilberti* from preserved specimens and therefore gave no color notes in the original description. In fact, the trout from the Kern illustrated by Evermann was atypical in both color and spotting. Of approximately 18 specimens at his disposal in 1904 at Stanford University, only three have the spotting pattern he considered representative of *S. a. gilberti*. The two specimens of *S. a. gilberti* at the Philadelphia Academy have spotting more like that of Evermann's *S. "whitei"* than that of Evermann's *S. a. gilberti*. Jordan (1925) considered *S. "whitei"* the form most closely related to *S. a. gilberti*. There is no evidence that trout in the Kern River and lower Little Kern River were isolated from each other or that there was not a free interchange of genes between these populations. A helicopter flight over the lower Little Kern River revealed that although the gradient is steep, there are no evident barriers to the movement of trout from the Kern River into the lower Little Kern River. Therefore, based on comparisons of specimens and the knowledge that *S. "whitei"* in the upper Little Kern basin was the result of a transplant of fish taken from the lower Little Kern River (the lower Little Kern River population was probably continuous with the Kern River population), it is concluded that *S. "whitei"* is a synonym of *S. a. gilberti*.

The original downstream distribution of trouts in the Kern and South Fork of the Kern rivers is not known. A series of streams north of Isabella Reservoir contain trout superficially resembling *S. a. gilberti*, but they are hybrids to various degrees between introduced *S. a. gilberti*, *S. a. aguabonita*, and *S. gairdneri*. Data of a sample from one of these brooks, Salmon Creek, are presented in Table 1. Salmon Creek was originally stocked with fish carried over from Trout Creek, a tributary to the lower South Fork of the Kern River (Houghton et al. 1889?). Typical *S. a. aguabonita* may have extended downstream in the South Fork of the Kern River to Trout Creek originally, but Ellis (MS 1915) and Waugh (1942) list the introduction as "rainbow trout." Hatchery rainbow trout were then stocked over a period of years (C. Ray personal communication).

Today, trouts in the Kern River and most of its tributaries are hybrids of recent origin. Several upper Little Kern River tributaries (upper Soda Springs, Wet Meadows, and Rifle creeks) contain essentially pure *S. a. gilberti*. When this trout was transplanted into the upper Little Kern drainage in 1875, it apparently filled the few suitable niches in these small swift streams and was better adapted than hatchery trout subsequently introduced. Gard and Seegrist (1965) discussed the persistence of resident genotypes after stocking with hatchery trouts. The main Kern River was a different situation. This large stream may be better rainbow trout habitat, and continual recruitment from introduced stocks and from new populations established in tributary lakes and streams resulted in increased hybridization.

The apparent intermediateness of *S. a. gilberti* between rainbow trout and *S. a. aguabonita* suggests a hybrid origin. The fact that some specimens of *S. a. gilberti* had basibranchial teeth prior to the introduction of cutthroat trout into the Kern River in 1895 and 1896 (Anonymous 1896) and comparisons with the undescribed red-banded trout collected in 1968 in northern California and southern Oregon (which bear a strong phenotypic resemblance to the Kern River trout) led us to doubt this theory.

More authoritative evidence that *S. a. gilberti* is part of the golden trout complex and not derived from a rainbow trout is based on its karyotype. In the summer of 1970, California Fish and Game Department personnel collected live *S. a. gilberti* in tributaries of the upper Little Kern River from populations we had determined to be essentially pure *S. a. gilberti*, and Dr R. Simon and Mr R. Wilmot, Oregon Cooperative Fishery Unit, Oregon State University, made chromosome preparations. They found a diploid number of 58 with 106 arms — identical to that of *S. a. aguabonita* (Table 1).

Speculation on Origins and Affinities

A discussion of the systematic status of *S. a. aguabonita* and *S. a. gilberti* must include the significant question of affinities of the golden trout to other western North American *Salmo*. Behnke (1970) mentioned similarities, particularly in the karyotypes, between *S. aguabonita* and the undescribed Apache trout of Arizona, and speculated on their relationships with *S. gilae* and *S. chrysogaster*. The similarities of these four trouts suggest a common origin associated with the lower Colorado River basin. If *S. a. aguabonita* originated from a trout radiating from the lower Colorado River system, the ancestral form probably gained access to California by annectent waterways between the lower Colorado River basin, Death Valley, and Owens Valley. Trout may then have entered the Kern drainage by headwater stream capture or, perhaps, by an ancient direct route of the Kern River into the Owens Valley as proposed by Lawson (1904). There is little doubt that fishes, native to Death Valley, of the genera *Rhinichthys*, *Cyprinodon*, and *Empetrichthys* were derived from the lower Colorado River Basin. The presence of *Rhinichthys*, *Catostomus* (*Pantosteus*) *santaanae* (Snyder), and *Gila* (*Gila*) *orcutti* (Eigenmann and Eigenmann) in southern California coastal streams reflects a former direct connection with the lower Colorado River drainage (Miller 1946, 1958; Hubbs and Miller 1948).

An alternative theory reversing this sequence of events can be proposed. In this instance, the direct ancestor of *S. a. aguabonita* was not derived from the lower Colorado River basin, but rather was the parental form that invaded the lower Colorado River system from the Kern area and gave rise to the Apache, Gila, and Mexican golden trouts.

We favor the opinion that *S. a. aguabonita* and *S. a. gilberti* did not diverge from a single ancestral progenitor in the Kern River basin. It is cautiously proposed that these trouts represent two independent invasions by already divergent forms of the golden trout complex. Speculation on origin and affinities of *S. a. gilberti* and on the position of the golden trout complex in the evolutionary heirarchy of western North American *Salmo* must take into account a group of trout assignable to neither *S. gairdneri* nor *S. clarki*. These trout, referred to as red-banded trout, are indigenous to desiccating basins in southern Oregon and to some tributaries of the upper Pit and McCloud rivers of the Sacramento River drainage in northern California (Behnke 1970). There is marked variability in meristic characters of these trout from different geographical areas, suggesting that they were isolated from each other for a long period of time. All populations, however, are characterized by yellow-golden body colors, a brick-red lateral band, often orange on the ventral region, distinct orange or yellow tips on the dorsal, anal, and pelvic fins, and a yellowish cutthroat mark.

The most impressive evidence of affinities between the red-banded and Kern River trouts is based on examinations of trout from Sheephaven Creek, a small tributary with barriers of the upper McCloud River near Mount Shasta. This trout bears a strong resemblance to *S. a. gilberti* in coloration, spotting, and meristic characters. Eleven of 21 specimens had basibranchial teeth, but most significant is the arrangement of dentition on the tongue (glossohyal). In addition to the normal salmonine single row of teeth on each side of the tongue, the specimens have a supplementary inner row anteriorly. This distinctive pattern has otherwise been found only in a few specimens of *S. a. gilberti*.

Speculation on a common ancestry between *S. a. gilberti* and the red-banded trout can also be based on the fact that both the Kern and Mount Shasta areas served as refugia during Pleistocene glaciation and that several plant and animal species are now common to these two disjunct areas. The foxtail pine (*Pinus balfouriana*) occurs only in the Sequoia and Shasta regions (Watts 1963), as do several other plants (Jepson 1923–25). Salamanders of the genus *Hydromantes* have a similar disjunct distribution (Stebbins 1954).

The distribution of trouts in the Kern River basin first encountered by man can be attributed to Pleistocene glaciation. Wisconsin Age ice covered the entire drainage of the Kern north of Golden Trout Creek on the east. At this time, Golden Trout Creek served as the outlet for the upper South Fork of the Kern River; the two streams separated at a much later date (Lawson 1904). On the west, glaciation covered the headwaters of the Little Kern River and all waters to the north (Matthes 1956, 1965). The southern extent of the Wisconsin ice is illustrated in Fig. 1. Relicts were left only south of the ice, and formation of barriers

on vacated waters prevented reinvasion by fish. Streams of the east slope of the Main Crest of the Sierras tributary to the Owens Valley were likewise glaciated. Later thermal periods and pronounced desiccation as discussed by Broecker (1965) and Morrison (1965) may explain why trout are not native to the Owens Valley in modern times.

We are probably approaching the limits of extracting meaningful information from orthodox taxonomic studies and zoogeographic interpretation regarding systematics of the golden trout complex. Analysis of karyotypes and biochemical taxonomy, primarily sequencing the amino acids of proteins, will play a major role in understanding evolutionary pathways of these forms. In any case, it now is apparent that the phylogenetic relationships among western North American *Salmo* are much more complex than commonly assumed.

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Spacing and Density in Schooling Threespine Sticklebacks (*Gasterosteus aculeatus*) and Mummichog (*Fundulus heteroclitus*)

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SYMONS, P. E. K. 1971. Spacing and density in schooling threespine sticklebacks (*Gasterosteus aculeatus*) and mummichog (*Fundulus heteroclitus*). J. Fish. Res. Bd. Canada 28: 999–1004.

Photographs of groups of threespine sticklebacks (*Gasterosteus aculeatus*) and mummichog (*Fundulus heteroclitus*) from above and from the side, and comparison of regularity of spacing at various densities with that in "schools" of dead fish arranged randomly or regularly, showed that live fish were usually spaced regularly when the average distance to nearest neighbour was less than 1 fish length. At greater distances (lower densities) spacing between fish became irregular. The lowest density at which regularity occurs could be considered the critical density separating potentially regularly spaced schools of fish from loose aggregations.

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ONE proposed characteristic of fish schools is regularity of spacing between individuals (Breder 1959). Breder (1954) has hypothesized that this regularity is brought about by an interaction between attractive and repelling stimuli. Attraction between fish must be counterbalanced at some point by repulsion if fish are not to move into contact with one another.

An alternative to Breder's hypothesis is that attraction between fish ceases when the fish are within some specific distance from one another. Spacing in such groups would approximate restricted randomness, regardless of the density of the group. Though observations by Cullen et al. (1965) make this alternative seem unlikely, it has not yet been experimentally eliminated.

The following observations and experiments were designed to discover whether spacing in groups of fish at different densities was regular or only appeared regular because of alignment of individuals, and to distinguish whether this regularity, if present, was brought about by behaviour of the fish, or was the result of chance arrangement in a limited space.

Materials and Methods

Two species of schooling fish, mummichog (*Fundulus heteroclitus*) and threespine stickleback (*Gasterosteus aculeatus*), were captured locally. The sticklebacks, all sexually mature, averaged 6.0 cm and ranged in size from 5.4 to 6.5 cm; the mummichog, some immature, averaged 6.7 cm and ranged from 5.1 to 7.5 cm.

About 50 of each species were placed, one species at a time, in a large plexiglass aquarium (200 × 48 × 35 cm). The fish formed dense aggregations when disturbed. Disturbance occurred during photographic sessions when I climbed a ladder next to the aquarium to reset one of the two cameras used to photograph behaviour. Fish were kept well fed, a condition favouring schooling among sticklebacks (Keenleyside 1955), and alignment was encouraged by creating a gentle current around the aquarium with a submersible pump. However, when the fish were left undisturbed for more than 4–5 hr, density of aggregation diminished. The time at which the last disturbance occurred prior to a photograph being taken was recorded so that the effect, if any, of disturbance on regularity of spacing in schools could be gauged.

To calibrate and refine available methods for measuring regularity of spacing, 35 dead mummichog were strung on individual wires and suspended in the area of the aquarium usually occupied by the schools of live fish. The dead mummichog were spaced regularly or randomly. For regular spacing, dead fish were hung at the corners of imaginary cubes with a fifth fish at the center, so that if the arrangement had extended indefinitely, each fish would have been as nearly as possible equidistant from four others. All regularly arranged fish were aligned as nearly perfectly as possible. For random arrangements the three spatial coordinates for each fish were chosen from a table of random numbers, and no effort was made to align the fish. Density was altered by restricting or increasing the space occupied by the grouping, and ranged over all but the lowest densities observed among live fish.

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Photographs were taken of the live and of the dead fish with two cameras simultaneously, one camera being at the side and the other overhead. Each camera was 1.8 m from the center of the aquarium. Twenty photographs of each species of live fish were taken, and these covered the whole range of behaviour from tight schools to undisturbed dispersed distributions (Fig. 1A–E). Since the null hypothesis was that spacing within apparently regular groups (schools) was random, only groups that were apparently regularly spaced (schooling) were selected for analysis from the complete range of densities (with the exception of one irregular clumped group retained for comparative purposes). At the lower densities (Fig. 1E) the fish did not appear to be schooling, but they were nevertheless apparently more regularly spaced in the groups selected than in others at similar densities. Not always was every fish visible in the photographs and some fish temporarily left the group (Fig. 1B) and were omitted from analysis. However, all groups of fish that were analysed contained more than 30 fish (31–53).

A second set of photographs was obtained of similar numbers (50) of sticklebacks and of mummichog in two outdoor ponds (oval, 4.6 × 9.1 m, depth 1.2 m; and circular, 7.0 m diam, depth 0.6 m). A series of six single photographs was taken from above the water at 5-min intervals for each species in each pond, one series at noon, the other at dusk (about 8:00 PM). Two of the 48 photographs had to be discarded because the fish were not visible due to surface ripples or darkness.

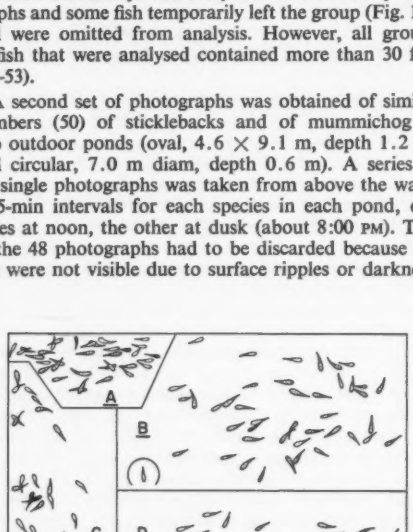

FIG. 1. Tracings from vertically oriented photographs of groups of sticklebacks, A and B, and mummichog, C, D, and E, showing the ranges of spatial distributions, and density analyzed. Deepest fish are indicated by open outlines, middepth fish by stippling, and those nearest surface by solid outlines. The circled fish (group B) was considered to be too distant from the others to be a part of the group.

Photographs from above the ponds were taken at the sounding of a bell set 5 min previously. The photographer was hidden, and, in contrast to the photographs taken of fish in the aquarium, fish in the ponds were undisturbed by people for at least 3 hr prior to being photographed.

Regularity of spacing of fish in the ponds could not be measured, but the photographs were compared visually with overhead photographs taken in the laboratory (specifically with Fig. 1A, B, and D) to decide whether spacing of fish in the ponds was of an apparently similar degree of regularity as that seen in the laboratory or not. The number of photos from the ponds showing a similar or greater degree of regularity then provided an independent estimate of the frequency with which regularity occurred among undisturbed fish.

Estimation of Density in the Aquarium

Density was estimated by dividing the number of fish in the group by the estimated volume of water occupied by the group. Volume was calculated by measuring the area of the school from the overhead photographs in fish lengths^2, and multiplying this by the average depth of the group measured from the horizontal photographs. Fish lengths were chosen as the unit of distance because the degree of crowding in groups of small or large fish is proportional to their length, not to absolute units such as centimeters. Difficulties in estimating volume in very irregular groupings were avoided by the selection of the most regular-appearing groups; in the most irregular of these, density was estimated by subdividing the group into more or less regularly shaped parts, calculating the volume of each part, and summing these to obtain the total volume. In the case of dead fish, the actual volume of the group was calculated by measuring distances between outermost fish, and this volume was checked against that calculated from the photographs. The estimate from the photographs was consistently about 1.5 times the actual volume. The estimated volumes of live fish groups were therefore corrected by this amount.

Estimates of Spacing

Two complementary methods of analyzing spacing were used. Both methods utilized "distance to nearest neighbour" expressed in fish lengths for estimating the form of the distribution. This distance was computed from the selected pairs of photographs in the following manner (Symons 1971): from the overhead photograph, the horizontal distance in the plane of the photograph (two dimensions) was measured from the nose of the fish under consideration to the nearest part of one or more neighbouring fish, using a pair of calipers and a ruler. From the photograph taken from the side, the vertical distance (one dimension only) between the same fish was measured in the same way. This distance measured on the photograph was converted to real distance in centimeters by multiplying with an appropriate correction factor. The three-dimensional distance between fish was then computed as the square root of the sum of the squares of the real distances. The average error

of 30 individual such estimates was found to be as little as 0.1–2.4 mm, or between 0.15 and 3.6% of 1 mummichog length (Symons 1971). For each group of fish, the mean distance to nearest neighbour and the variance were calculated. These two data formed the basis for the two methods of estimating regularity of spacing.

The first method of estimating regularity of spacing utilized the variance of the mean distance to nearest neighbour (developed from a method of Taylor 1961). Since in a Poisson distribution the variance equals the mean, the ratio between the variance and mean (variance ratio) of randomly distributed fish was expected to be constant at different densities. When tested against random and regular distributions of dead fish this expectation proved justified over the range of densities employed ($r = -0.419$ and $+0.047$ for randomly and regularly arranged dead mummichog, respectively, $P > 0.05$). The possible effect of density on the variance ratio was therefore ignored.

To estimate the importance of behavioural interaction in bringing about increased regularity of spacing among schooling fish, the variance ratio was calibrated in terms of maximum regularity obtained with the arrangements of dead fish. The average variance ratio for regularly arranged dead fish was 0.51; the lowest ratio obtained (=100% regularity) was 0.35. The average variance ratio for randomly arranged dead fish was 1.80 (=0% regularity). If a group of live fish were to have a variance ratio, for example, of 1.08, which is halfway between 0.35 and 1.80, its regularity would have been 50%. Calculation of percentage clumping was not possible because the variance ratio for maximally clumped fish was unknown. Therefore, the variance ratio was retained for expression of the degree of clumping. The variance ratio is not normally distributed and therefore no attempt was made to set confidence limits.

The second method of estimating regularity of spacing was developed from Clark and Evans (1954). They used the ratio of the observed mean distance to nearest neighbour (R_A) to the estimated mean distance at that density (R_E) when the distribution was assumed to be random ($R_E = \frac{1}{2\sqrt{\rho}}$, where ρ = density when measured in the same units used to measure R_A). Theoretically, in two dimensions, when $R_A:R_E = 1$, the distribution is random; regular spacing is indicated by ratios greater than 1 up to a maximum of 2.149, obtained with a perfect hexagonal arrangement (in three dimensions the maximum is probably something less than this); clumping is indicated by ratios less than 1 to a minimum of zero, which is reached when observed distance to nearest neighbour becomes zero.

In practice, these theoretical values were seldom or never attained with the random and regular arrangements of dead fish, mostly because distances between fish were measured from nose to nearest part of nearest neighbour. The Clark and Evans measure did vary with density (Fig. 2, $r = 0.732$ and -0.830 for random and regular groups, respectively, $P < 0.05$) so that the ratio $R_A:R_E$ for random arrangements of dead fish = $0.145 \times$ density $+1.020$ (the equation by least squares for the lower line in Fig. 2[1]). The ratios from fish arranged with maximum regularity varied with density so that $R_A:R_E = -0.185 \times$ density $+1.787$. If the line were truly to represent maximum regularity, however, all points had to fall below it. By increasing the constant 1.787 to 1.910, the resulting line (uppermost in Fig. 2) passed through the highest point and therefore approached and was taken to represent 100% regularity. The line through random points represented 0% regularity. The percentage regularity for groups whose ratios fell between these two lines was calculated as for the variance ratio; that is, the percentage regularity for a group falling between the 0 and 100% lines was calculated as the distance above the lower line divided by the total distance between the lines at that density multiplied by 100. For groups having clumped distributions, the ratio itself was used. The distribution of $R_A:R_E$ is approximately normal (Clark and Evans 1954).

The measures were complementary because irregularities in the distribution of fish had different effects on the two measures. For instance, if a group of fish were made up of pairs of evenly spaced fish, not necessarily regularly spaced from other pairs, the variance ratio would be less than that of a random distribution, indicating regular spacing, but the Clark and Evans ratio would also be less than that of random distributions, indicating clumping. In this example, clumping does

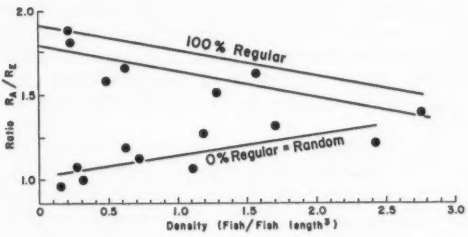

FIG. 2. The measure of group spacing, ratio $R_A:R_E$, applied to groups of randomly and regularly arranged dead mummichog, was affected by the density of the grouping. The two lower lines were calculated by least squares, the lower from randomly spaced and the upper from regularly spaced fish. The uppermost line is parallel to the line immediately below it and passes through the point representing maximum regularity (100%) attained with dead fish. Below 0% regularity (random spacing) the spacing becomes clumped.

[1]If sufficient data were available, the line through points for random groupings would be bowed, with the end at high densities depressed. For this reason, and also because the ratio $R_A:R_E$ and density (ρ) are not independent, ρ being used in the derivation of the ratio, calculation of a linear regression, strictly speaking, is not justified. However, regardless of whether this base line is drawn freehand bowed or straight, or is calculated by least squares, the conclusion drawn remains unaltered.

exist because the whole group consists of subgroups of two fish. To emphasize the complementary nature of the measures, I have called the variance ratio a measure of individual spacing, and the Clark and Evans ratio a measure of group spacing. When both measures indicate regularity, the group is homogeneously regular with no subgroups or clumps.

Dependence of Regularity of Spacing on Density

Individual spacing in some groups of mummichog and sticklebacks was found to have a regularity as high as 90% of the maximum attained with regularly arranged dead fish (Fig. 3). Group spacing was less regular, the maxima being 85% for sticklebacks and 40% for mummichog (Fig .4). However, despite the selection of the most regular-appearing groups, neither individual nor group spacing was regular in groups whose density was less than 0.3 fish/fish length^3. The average distance between nearest neighbours expected in a random distribution (R_E) at this density is approximately 1 fish length ($\frac{1}{2}\sqrt{0.3} = 0.91 = R_E \approx 1$).

Although disturbance generally caused fish to aggregate at higher densities than when left undisturbed for some hours, there was no significant ($P > 0.10$) relation in the selected sample of photographs between recency of disturbance and density, or between recency of disturbance and either of the two measures of spacing (chi-square tests).

The question now arises as to whether the degree of regularity displayed was significantly different from a random sampling of randomly spaced fish. If the spacing had been random, about 50%

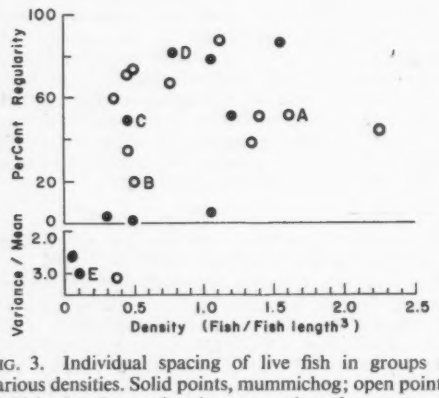

FIG. 3. Individual spacing of live fish in groups of various densities. Solid points, mummichog; open points, sticklebacks. Lettered points are values for groups in Fig. 1.

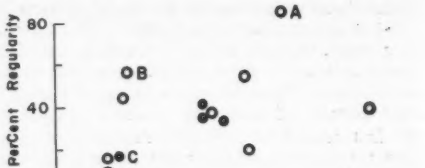

FIG. 4. Group spacing among live fish in groups of different densities. Groups were chosen for apparent regularity except that at 1.55 fish/fish length^3. Solid points, mummichog; open points, sticklebacks. Lettered points are values for groups in Fig. 1.

of the selected groups of live fish should have fallen above and 50% below any line, such as that in Fig. 2, which bisected the field of points representing random arrangements of dead fish. Significantly ($P = 0.04$, binomial test) more groups of live fish fell above this line than below it (Fig. 3; the point at the density 1.55 fish/fish length^3 was not counted as this group was chosen for irregularity). Rejection of the possibility that this is a random sampling of randomly spaced fish leaves three alternatives: (1) spacing in groups of fish is usually random, but the observer can recognize and select groups with regular spacing when by chance regularity occurs; or (2) fish are usually regularly spaced in groups but regularity cannot be recognized by an observer; or (3) schooling fish are usually regularly spaced, and an observer can distinguish between regular and irregular spacing. Since in the second and third alternatives regular spacing must be brought about by behaviour of the fish, only the first alternative remains to be eliminated to show that regularity is caused by behaviour and does not arise by chance. According to the first alternative, an observer can recognize and select groups with regularity of spacing. Chance regularity in schools of randomly spaced fish would occur in 5% or less of unselected independent photographic samples. The frequency of this recognizable regularity was therefore examined next.

Frequency of Recognizable Regularity

Groups of fish photographed in the outdoor ponds were subjectively appraised as having regular spacing between individuals by comparing the

outdoor photographs with the overhead photographs of groupings A, B, and D (Fig. 1) taken in the laboratory. Regularity of equal or greater degree to that in groups A, B, and D was judged to occur in almost half of the photographs taken in the outdoor ponds:

Photographs showing:	Regularity	No regularity	Total
Sticklebacks	6	18	24
Mummichogs	15	7	22

Within the 5-min period between photographs, groups of fish could and frequently did traverse the entire lengths of the ponds, the groups changing shapes and the fish their positions as they proceeded. This was taken as good evidence of statistical independence between observations taken at 5-min intervals. In addition, the use of photographs of different fish in the two ponds necessitated independence between some samples.

Among independent samples of groupings, only 5%, or two photographs of the 46, should have shown recognizably regular spacing of the degree occurring in groups A, B, and D (Fig. 1). No statistical tests are needed to show that the proportion of photographs showing schooling is truly more than 5%. Therefore, providing that the assumption that regularity can be subjectively recognized is correct, regular spacing is so frequent that it must be brought about by the behaviour of the fish. If the assumption is incorrect, i.e., an observer cannot correctly identify regularity the regular spacing must still be brought about by the behaviour of the fish according to alternatives (2) and (3) above. Therefore, regular spacing is brought about by behaviour and does not arise by chance.

General Discussion

The main finding of this work is that the two species studied, when separated by 1 fish length (0.3 fish/fish length^3), or more, are spaced at random or in clumps, but when within 1 fish length they often become regularly spaced. Spacing between nearest individuals may always be regular at densities above 0.4 fish/fish length^3, because even the group that was selected for its clumped appearance (and that was clumped according to the group measure) had regular spacing according to the individual measure (group at density 1.55, Fig. 3). This regularity was shown to be caused by behaviour of the fish; it did not arise by chance.

Breder (1954) hypothesized that regularity of spacing is brought about by interaction of attraction and repulsion between fish. When spacing is not regular, either attraction or repulsion is effectively absent or varies in strength from fish to fish.

The simplest explanation for lack of regular spacing at low densities is that attraction decreases and the fish disperse, so that repulsion in turn decreases. With reduced attraction and repulsion, movement caused by other factors (e.g., search for food) disrupts the regular spacing. These groups would more properly be called aggregations than schools (Breder 1959).

At densities higher than 0.3 fish/fish length^3 attraction and repulsion may be having a significant effect on spacing, but spacing may be irregular because attraction (or repulsion) varies from individual to individual. Such groups still fall within the concept of a "school" caused by mutual attraction between fish, even though Breder (1959) excludes irregularly spaced groups. Further, spacing may or may not be judged as regular depending upon whether or not the whole or part of the group is analysed. For example, the group at density 1.55 fish/fish length^3 was clumped according to the group measure, but had regular spacing between individuals in the clumps according to the individual measure. Therefore, the generally accepted empirical criterion that a school is a group of fish brought together at high density for no other apparent reason than mutual attraction, which theoretically implies but does not necessitate regular spacing, is probably the most useful both in practice and in theory.

The feature separating schools from aggregations, then, is the density at which attraction and repulsion interact sufficiently to have the potential for regulating spacing. The present work suggests this density can be defined as the highest density below which spacing is always random or clumped. For threespine sticklebacks and mummichog, this critical density appears to be 0.3 fish/fish length^3.

The lowest density with regular spacing (critical density) will be reached when attraction and repulsion are maximal in relation to disruptive forces. This maximized relation may best be reached among a slightly frightened group of fish that is accelerating its swimming speed, because fright appears to increase attraction (it usually increases density) and it may also indirectly increase repulsion if repelling stimuli originate from the tail beat, the amplitude of which is increased during acceleration (van Olst and Hunter 1970).

Acknowledgments

I thank A. Sreedharan for correcting several mathematical blunders in earlier drafts of this paper.

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Genetics of Multiple Malate Dehydrogenase Isozymes in Skeletal Muscle of Walleye (*Stizostedion vitreum vitreum*)[1]

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CLAYTON, J. W., D. N. TRETIAK, AND A. H. KOOYMAN. 1971. Genetics of multiple malate dehydrogenase isozymes in skeletal muscle of walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Bd. Canada 28: 1005–1008.

Electrophoresis of extracts of white skeletal muscle revealed a total of six phenotypes of malate dehydrogenase (MDH) (L-malate; NAD oxidoreductase, EC 1.1.1.37) isozymes in walleye (*Stizostedion vitreum vitreum*). The individual MDH phenotypes consisted of four or more MDH isozymes.

The heritability of all six phenotypes was tested in a breeding experiment. The results of the breeding experiment provided evidence for the existence of three nondominant alleles at one MDH locus. Subcellular fractionations indicated that these three alleles code for a supernatant (extramitochondrial) form of MDH. The frequencies of these alleles varied in fish from four locations in western Canada.

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A search for biochemical characters that would distinguish populations of walleye (*Stizostedion vitreum vitreum*) (order Perciformes, family Percidae) led to the discovery of multiple isozymes and the presence of several phenotypes of malate dehydrogenase (MDH) (L-malate; NAD oxidoreductase, EC 1.1.1.37) in this species. These initial observations have been pursued in a number of ways. This paper describes the results of a breeding experiment that tested the heritability of the six known walleye MDH phenotypes. The geographical distribution of walleye MDH phenotypes from four locations in western Canada is also presented.

Materials and Methods

Wild fish utilized in this study were collected during 1970 from Lake Winnipeg in the vicinity of Grand Rapids, Lake Winnipegosis, near Duck Bay, and also from West Blue Lake in the Duck Mountain Park. These lakes are all in Manitoba.

The parental fish utilized in the breeding experiment were from Waskesiu Lake, Prince Albert National Park, Saskatchewan. These fish were trapped on their normal spawning grounds in the Kingsmere River. Forty matings were made from 63 individual fish on May 12, 1970. The eggs were water hardened in floating mesh boxes in the river overnight then transferred to a hatchery in the Prince Albert National Park. The incubation was continued in the park hatchery until June 2, 1970.

The parental fish were killed after spawning, iced immediately, and shipped to the Freshwater Institute at Winnipeg. Subsequently all the parental fish were analysed for MDH phenotype. On the basis of this analysis egg lots from 20 matings were selected for hatching and subsequent study of the MDH phenotypes in the progeny.

The selected egg lots were shipped to the Freshwater Institute on June 2, 1970. Incubation was continued in flowing tap water at the prevailing temperature, but with the addition of sodium thiosulphate to reduce any free chlorine. The larval fish hatched during the period June 5–15, 1970, at a water temperature of 10–12 C. The fish were maintained in the laboratory through June, July, and August on a mixed diet of brine shrimp nauplii, hard boiled egg yolk, and plankton collected from local ponds. The water temperature reached a maximum of 21 C on August 14. During August the fish attained a convenient size and many were killed for determination of MDH phenotype. The fish remaining by August 25 were killed and frozen for subsequent MDH analysis.

Tissue extracts were prepared for electrophoresis by homogenization of muscle tissue with a distilled water solution of nicotinamide adenine dinucleotide (NAD), 300 mg/liter, in a Teflon and glass tissue grinder. Adult

[1]Presented in part at 32nd Midwest Fish and Wildlife Conference, Winnipeg, Man., December 6–9, 1970.

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fish extracts were prepared from white muscle in the ratio 1 g tissue/2 ml extraction solution. Prior to electrophoresis these extracts were clarified by centrifugation at 25,000 *g* and 2 C. Extracts of the juvenile fish were prepared from the portion of the fish that remained after removal of the head and internal organs. These samples were homogenized in Teflon and glass tissue grinders at approximately a 1:3 ratio of tissue to NAD solution. These extracts were clarified by centrifugation at 7000 *g* and 2 C.

Mitochondrial and supernatant extracts were prepared by the procedures of Bailey et al. (1969), with the substitution of 3-mercapto-1,2-propanediol for mercaptoethanol in the present work.

Starch-gel electrophoresis (Tris[2]-citrate buffer *p*H 8.0) and isozyme visualization was carried out as described by Clayton and Gee (1969) with the substitution of malic acid for lactic acid in the present work. The electrophoresis of mitochondrial preparations was conducted similarly except that the buffer utilized was bis–Tris[3] at *p*H 6.5 (Lewis 1966). The *p*H was adjusted at 22 C, although electrophoresis was conducted at 2–3 C.

Results and Discussion

Six phenotypes of MDH isozymes were revealed by electrophoresis of white muscle extracts followed by specific staining (Fig. 1). No staining indicative of dehydrogenase was observed when malate was deleted from the stain solution. We have developed a system of nomenclature to aid in the discussion of these phenotypes and their constituent isozymes (Fig. 2). Our system is based on the assumption that the isozymes are dimers, composed of two polypeptide chains each under independent genetic control, in accord with the observations of Bailey and Wilson (1970). Additional evidence for the dimeric nature of MDH isozymes in organisms other than bacteria and fungi has also been presented by Murphey et al. (1967) and Kitto (MS 1966).

Malate dehydrogenase usually exists in two forms: mitochondrial MDH, found chiefly within mitochondria, and supernatant MDH, found within the general cell cytoplasm (Thorne et al. 1963). Walleye MDH isozymes were tested to see into which of these forms they could be classified. Figure 3 shows a comparison of the MDH isozymes in mitochondrial, supernatant, and whole tissue extracts made from the white muscle of a C^2C^2 MDH walleye. A further comparison of mitochondrial MDH isozymes from heart, red muscle, and liver with white muscle, whole tissue, and supernatant extracts is shown in Fig. 4. These latter preparations were made from the tissues of a C^2C^2 walleye and the electrophoresis was conducted at *p*H 6.5 in a bis–Tris–citrate buffer. This buffer seems to preserve the enzymatic activity of the mitochondrial MDH isozymes more effectively than the *p*H 8.0 Tris–citrate system.

Some of the minor bands that have frequently been found in MDH electropherograms (Kitto MS 1966) appear under these conditions. The two least anodal, high activity isozymes (AA and BB in Fig. 2) contain considerable mitochondrial MDH activity, whereas the isozymes that distinguish phenotypes (BC and CC in Fig. 2) appear mainly in whole extracts or supernatant preparations and are probably supernatant MDH.

We suspect that the BB isozyme in whole tissue extracts is composed of both mitochondrial and supernatant MDH that exist independently and are not resolved electrophoretically under our conditions. We therefore further assume that BC isozymes are supernatant isozymes produced by combination of B and C supernatant subunits coded for independently at two distinct supernatant MDH loci. These points are under investigation at the present time. Our general interpretation follows that of Bailey and Wilson (1970) who suggest that many fish species have duplicated loci for both supernatant and mitochondrial MDH. Additional evidence for the general multiplicity of MDH isozymes in fish has recently been presented by Aghasadeh and Ritter (1971).

The various MDH isozymes are not produced in constant relative amounts in all tissues. Extracts from a C^2C^2 MDH walleye show the presumed mitochondrial isozymes AA and BB to predominate in heart, red muscle, and liver whereas the phenotype distinctive CC isozymes are moderately active in only white muscle (Fig. 5). A generally similar variation in tissue specificity of MDH isozymes in salmonids was observed by Bailey and Wilson (1970).

The assignment of genotypes to the phenotypes that was shown in Fig. 2 was tested by means of a breeding experiment as outlined in Table 1. Electropherograms derived from extracts of progeny from the breeding experiments are shown in Fig. 6. The matings shown in Table 1 are the 15 that produced usable progeny of the 20 that were selected in the manner described under methods. The results show substantial agreement with simple Mendelian genetics for the MDH phenotypes in walleye. Thus matings 1 and 2 show C^1C^1 breeds true, whereas mating 3 shows that $C^2C^2 \times C^1C^1$ gives only the expected C^1C^2 progeny. All of the heterozygous matings (4–9) of the type expected to yield only

[2]Tris = tris (hydroxymethyl) amino methane.

[3]bis–Tris = bis (2-hydroxyethyl) imino–tris (hydroxymethyl) methane.

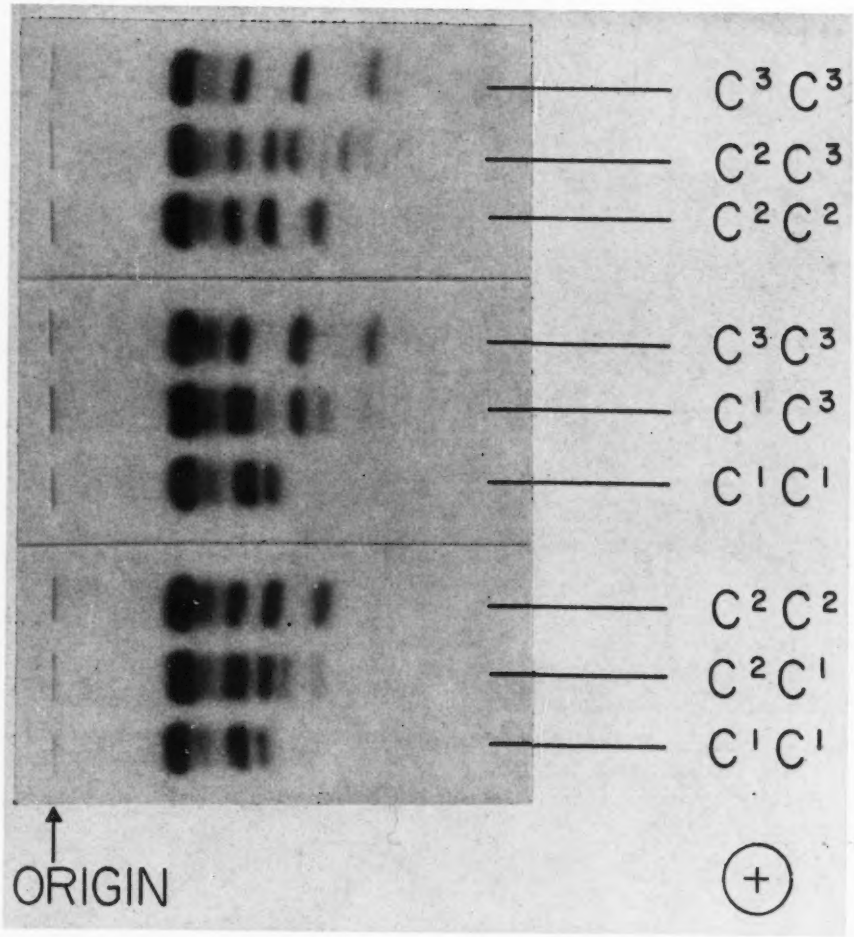

FIG. 1. Electropherograms of adult walleye white muscle MDH isozymes, showing the six known phenotypes.

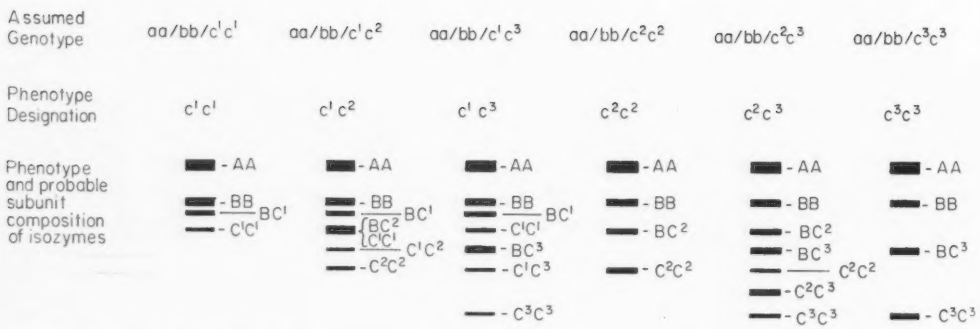

FIG. 2. Diagram of walleye MDH phenotypes showing assumed genotype and probable subunit composition of isozymes.

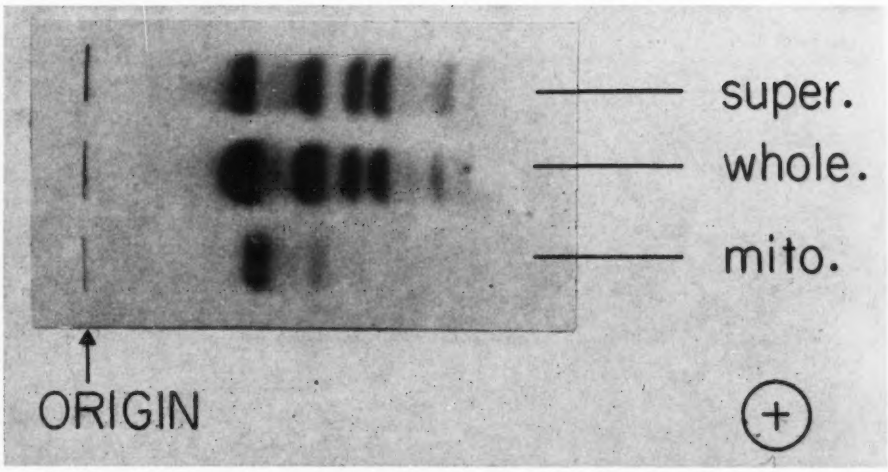

FIG. 3. Electropherograms of MDH isozymes in supernatant, whole tissue, and mitochondrial fractions of white muscle from a C^2C^3 walleye.

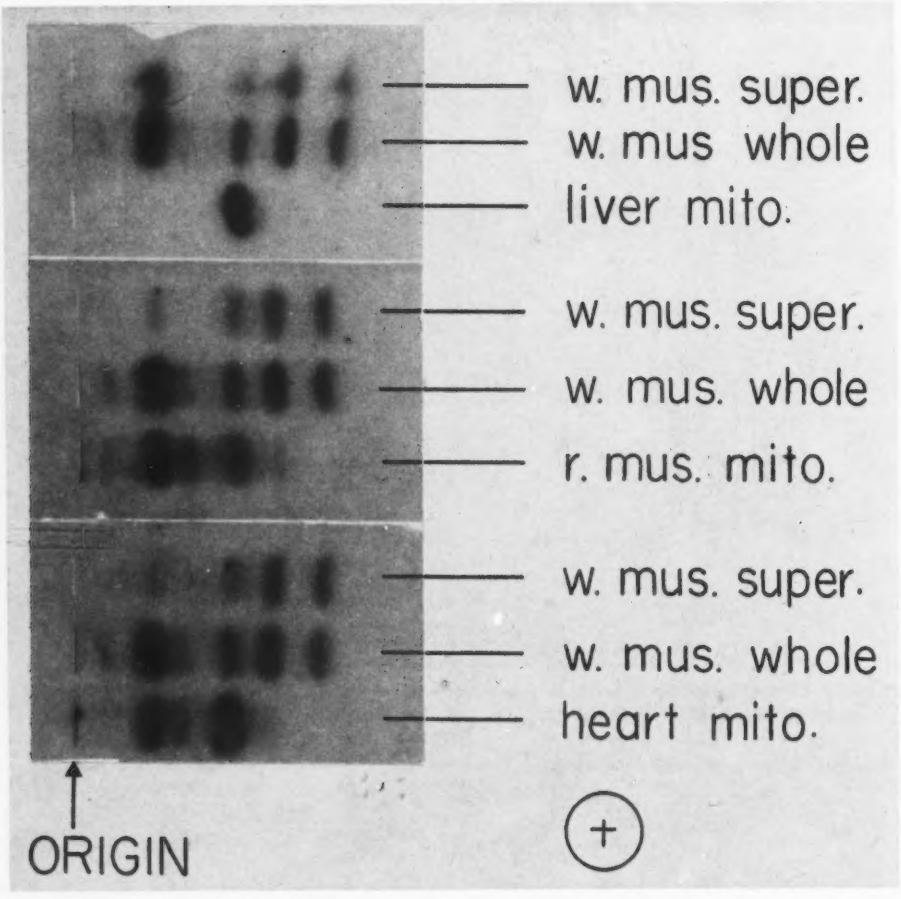

FIG. 4. Electropherograms of MDH isozymes in mitochondrial, whole tissue, and supernatant preparations from a C^2C^2 walleye.

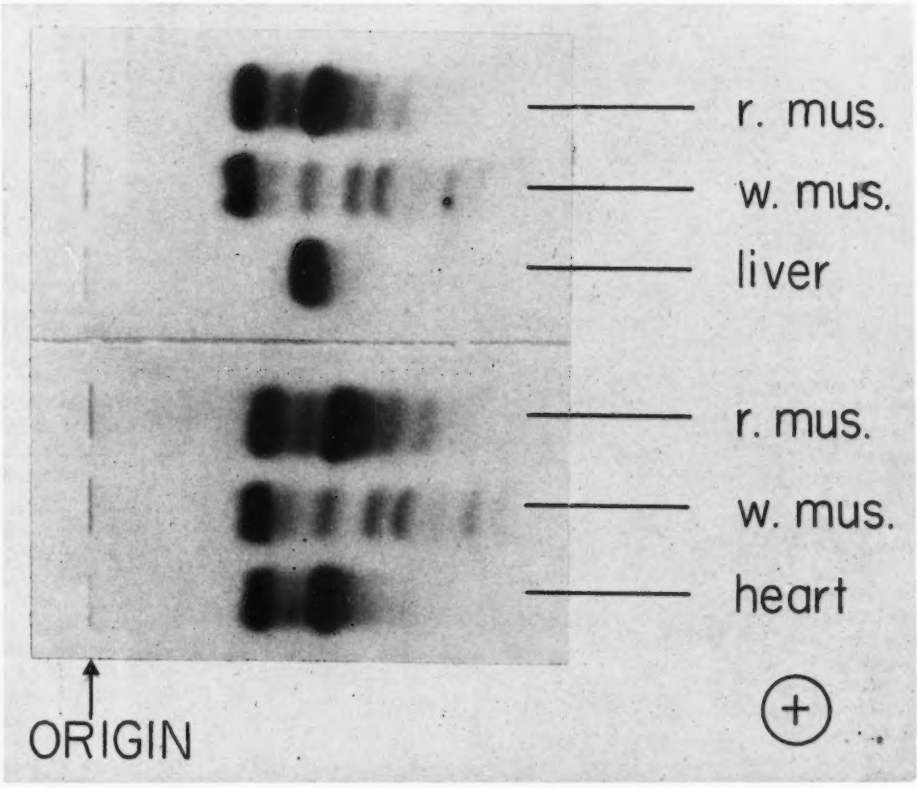

FIG. 5. Electropherograms of MDH isozymes in various tissues of a C^2C^3 walleye.

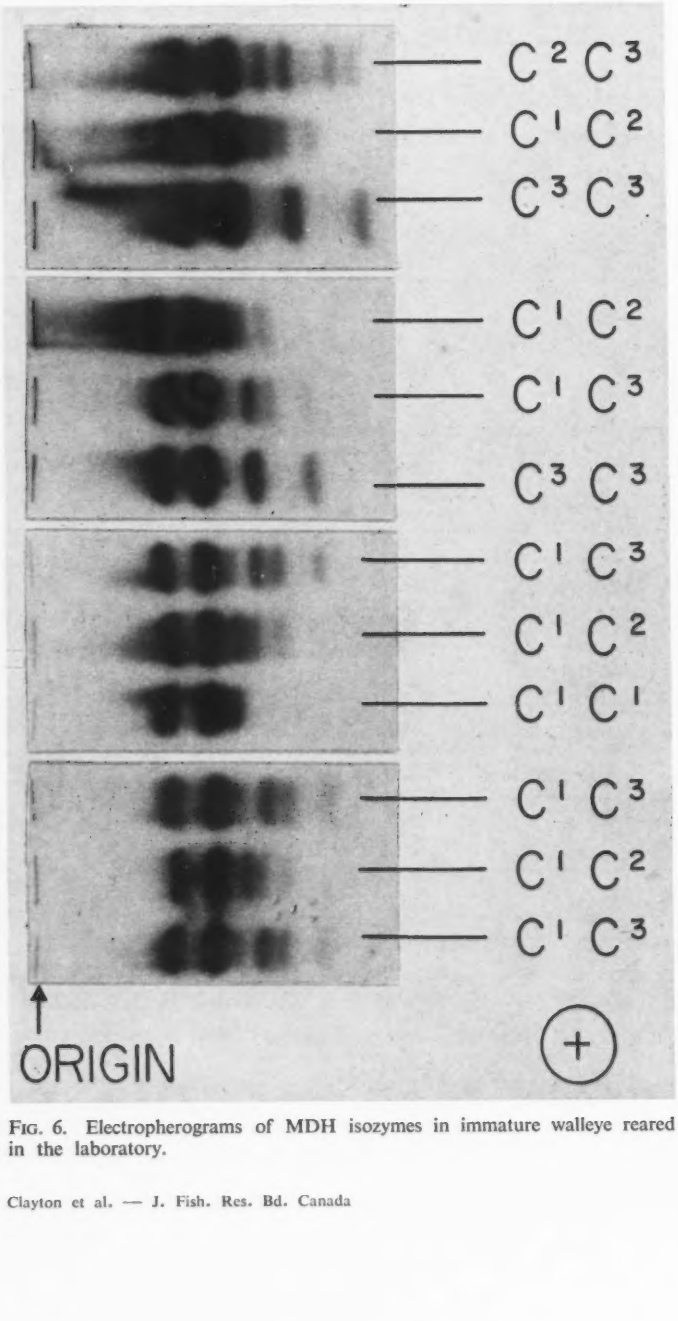

FIG. 6. Electropherograms of MDH isozymes in immature walleye reared in the laboratory.

TABLE 1. Inheritance of malate dehydrogenase phenotypes in walleye.

Mating[a]	Parental phenotype		No. progeny Phenotypes					χ^2	P[b]
	Male	Female	C^1C^1	C^1C^2	C^1C^3	C^2C^3	C^3C^3		
1	C^1C^1	C^1C^1	37					0	1.0
2	C^1C^1	C^1C^1	49					0	1.0
3	C^2C^2	C^1C^1		63				0	1.0
4	C^1C^3	C^1C^1	42		36			0.46	0.5
5	C^1C^1	C^1C^3	24		25			0	1.0
6	C^1C^1	C^2C^3		45	56			1.20	0.3
7	C^3C^3	C^1C^3			44		50	0.38	0.5
8	C^1C^3	C^3C^3			41		28	2.45	0.15
9	C^2C^2	C^3C^3				34	32	0.06	0.8
10	C^1C^3	C^1C^3	27		58		24	0.61	0.75
11	C^1C^2	C^1C^3	20	32	15	27		7.19	0.06
12	C^1C^2	C^1C^3	43	61	45	64	3[c]	6.55	0.09
13	C^1C^2	C^1C^3	32	23	30	32		1.87	0.6
14	C^2C^3	C^1C^3		15	20	28	20	4.18	0.25
15	C^2C^3	C^1C^3		48	76	48	44	12.15	0.008

[a]A common female parent was used in matings 1 and 3; 2 and 4; 5 and 14; 7, 10, and 12; 8 and 9.
[b]Probability of worse deviation from expected simple Mendelian ratios of numbers of progeny.
[c]These are likely strays from matings 7 or 10 and are not considered in probability calculations.

two classes of progeny and the single mating (10) expected to give three classes of progeny produced results in substantial agreement with the Mendelian expectation.

In double heterozygous matings expected to yield progeny of four different phenotypes (matings 11–15) the results were more variable. Thus, the results of mating 13 were in good agreement with expectation, whereas one replicate, mating 11, yielded a deficient number of C^1C^3 progeny and the other replicate, mating 12, yielded deficiences of both C^1C^1 and C^1C^2 progeny. On the other hand C^1C^2 progeny were by far the most abundant in mating 15. From the scatter of these observations it seems unlikely that our results show any differential mortality of any one phenotype, although the observed approximately 100-fold mortality in each of our experimental lots could have provided a mechanism for the operation of a differential mortality between the various phenotypes.

The delayed activation of paternal alleles has been observed in other studies (Bailey and Wilson 1970; Goldberg et al. 1969; Hitzeroth et al. 1968). It is pertinent to examine the present results to see if some of the variation could be accounted for by this means. Table 1 shows that in matings 11–15 the distinctive paternal gene was c^2. In matings 13 and 15 there were slight deficiencies of C^2 progeny that are, however, statistically insignificant, whereas in matings 11, 12, and 14, a slight excess of C^2 progeny was found.

We conclude from these considerations that the results observed do not show significant deviation from the expected Mendelian ratios of progeny, and, further, we conclude that the breeding data support the phenotype/genotype assignment shown in Fig. 2. We interpret our results as evidence that the walleye we have examined have three nondominant alleles at the locus for the most anodal form of supernatant MDH.

The distribution of these MDH genes in some natural walleye populations was investigated. Table 2 shows the numbers of MDH phenotypes found in walleye from some western Canadian waters. The expected numbers of phenotypes were calculated from a binomial expansion of the observed gene frequencies, based on our assignment of genotype as shown in Fig. 2. The close agreement between expected and observed numbers in most samples may be considered as further evidence in support of our assignment of genotypes to the observed phenotypes. The sample from Lake Winnipeg at Grand Rapids does not show good agreement between the observed and expected numbers of fish of each phenotype. This may in part be a result of the small sample size of only 33 fish. This lot and the lot from the Red River are included mainly to show that the c^1 gene, although rare, does occur at several locations in addition to Waskesiu Lake.

The wide range in these MDH gene frequencies that occurs in natural populations, as shown in Table 2, suggests the possibility of utilizing this

TABLE 2. Distribution of malate dehydrogenase phenotypes and gene frequencies in western Canadian walleye.

Location		Phenotype						Gene frequency		
		C^1C^1	C^1C^2	C^1C^3	C^2C^2	C^2C^3	C^3C^3	c^1	c^2	c^3
Red R.	obs.[a]	–	–	1	15	5	8	0.017	0.534	0.448
(Lockport, Man.)	exp.	0.01	0.5	0.4	13.9	5.8	8.3			
Waskesiu L.	obs.	18	10	24	6	4	1	0.556	0.143	0.302
(Kingsmere R.)	exp.	19.4	10.0	21.1	5.4	5.7	1.3			
L. Winnipegosis	obs.	–	–	–	45	31	24	0.0	0.465	0.535
(Duck Bay)	exp.	–	–	–	49.7	28.6	21.6			
West Blue L.	obs.	–	2	–	38	13	39	0.011	0.641	0.348
	exp.	0.01	1.3	0.7	41.0	11.1	37.8			
L. Winnipeg	obs.	–	3	–	21	2	7	0.045	0.576	0.379
(Grand Rapids)	exp.	0.1	1.7	1.1	14.4	4.7	10.9			

[a]obs. = number observed; exp. = number expected.

variation in walleye management. The common management practice of planting walleye in waters that contain depleted natural populations could be readily evaluated by choosing a suitable stock that has an MDH gene frequency sufficiently distinct from that of the natural population. An analysis of MDH gene frequencies of small fish some months after the planting should thus show whether there had been an appreciable survival rate of the introduced fish.

Acknowledgments

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Discreteness of Atlantic Herring (*Clupea harengus harengus*) Populations in Spring and Autumn Fisheries in the Southern Gulf of St. Lawrence

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MESSIEH, S. N., AND S. N. TIBBO. 1971. Discreteness of Atlantic herring (*Clupea harengus harengus*) populations in spring and autumn fisheries in the southern Gulf of St. Lawrence. J. Fish. Res. Bd. Canada 28: 1009–1014.

Evidence was obtained that spring and autumn herring fisheries in the southern Gulf of St. Lawrence are supported by two discrete stocks. Samples from the two fisheries taken in 1965 through 1969 differed in length- and age-frequency distributions, year-class composition, growth rate, spawning time, and mean numbers of pectoral and dorsal fin rays.

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SPRING- and autumn-spawning populations of Atlantic herring (*Clupea harengus harengus*) support major commercial fisheries in the Gulf of St. Lawrence. Until 1966 both fisheries were based on prespawning and spawning aggregations of herring and were carried on with gillnets and traps set close to the coast. An abrupt change in these fisheries occurred after the introduction of purse seining. The seasons and areas of operations were expanded, and the catch increased from less than 100 million lb (45,360 metric tons) to more than 300 million lb (136,079 metric tons) (Fig. 1). This sharp increase in catch raised questions as to whether the population as a whole could support the increased rate of exploitation, and whether the fishery was exploiting more than one stock.

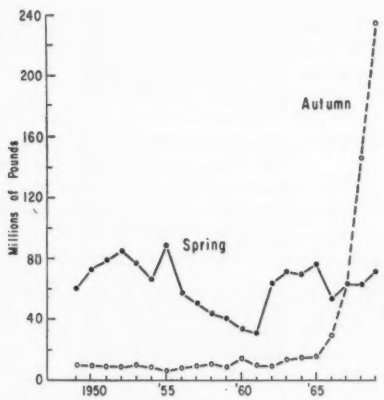

FIG. 1. Annual landings of herring from spring and autumn fisheries in the Gulf of St. Lawrence (1949–69).

There have been differences of opinion about whether spring- and autumn-spawning herring in the Gulf of St. Lawrence are discrete stocks. Perley (1852) and Prince (1907) assumed that there were two separate stocks. Tibbo (1957) and Day (1957) considered the stocks separate but were unable to provide convincing evidence of discreteness. Jean (MS 1956) hypothesized a single stock in which each spawning group was a mixture of spring- and autumn-hatched fish in similar proportions. It seemed desirable, therefore, to reexamine the biological characteristics of herring in the area, in an attempt to answer the question of the interrelation of the stocks that support these two fisheries.

The study included comparisons of lengths, ages, growth, maturation stages, and meristics of samples of herring taken from the two fisheries.

Materials and Methods

Length, age, sex, and maturity data were collected from more than 13,500 fish landed by commercial fishermen at nine major fishing ports (Fig. 2) during the years 1965–69. Most of the examinations were done while the fish were fresh. In 1969, the sampling program was expanded to include vertebral counts and dorsal and pectoral fin-ray counts.

Samples for all years were separated into spring and autumn categories on the basis of sampling dates. Since the spring fishery usually ended by mid-June, and the autumn fishery did not start before mid-July, June 30 was taken as the separation date.

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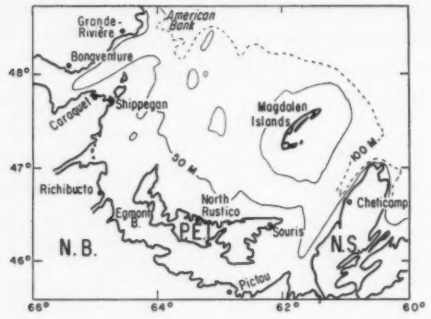

FIG. 2. The southern Gulf of St. Lawrence, showing major fishing ports where herring samples were collected.

Lengths were measured from the tip of the snout to the end of the longer lobe of the caudal fin. They were recorded in millimeters and grouped in 1-cm intervals, i.e., lengths of 200–209 mm were included in the 20-cm group.

Ages were estimated from otoliths and recorded as the number of winter (hyaline) rings. Fish more than 9 years old were not assigned specific ages but were grouped as >9. The otolith margin was included as an annulus for the spring but not for the autumn samples. The appearance of the otolith nucleus (hyaline or opaque) was not taken into consideration for separating spawning types, since Messieh (1969) showed this technique to be suspect.

The chi-square test was used to examine the differences in length- and age-frequency distributions between fish from spring and autumn fisheries. The hypothesis was that the samples were drawn from the same population, and the 0.01 probability value was used as the criterion for rejecting it.

For growth rates, lengths were back-calculated from scales by Lee's method (Messieh 1969). Growth curves were fitted to mean back-calculated lengths by the von Bertalanffy equation (below) using Allen's (1966) method.

$$L_t = L_\infty (1 - e^{-k(t-t_0)})$$

where L_t = body length estimated for time t; L_∞ = theoretical maximum length; k = coefficient of growth; t_0 = theoretical time when body length is zero.

The following values for the three parameters were used to plot the growth curves:

	k	t_0	L_∞
Spring fishery			
1966	.525	−.015	32.28
1967	.514	−.015	32.17
1968	.474	−.084	33.17
Autumn fishery			
1967	.524	−.277	33.89
1968	.533	.189	33.95

Maturation stages were recorded using a simple system with four stages. Comparison with the more complex system recommended by the ICES Herring Committee (Anon. 1962) is given in Table 1.

Vertebral and fin-ray counts were made from radiographs.

Length and Age Composition

Percentage length distributions of herring (Fig. 3) from the two fisheries were different in all years. Except for the 1969 samples, herring in the autumn fisheries were significantly larger than those in the spring fishery. The differences in mean lengths (Table 2) were obvious.

Age-frequency distributions (Fig. 4) and mean ages (Table 2) in the two fisheries differed in all years. In the spring fisheries, the 1959 year-class was dominant in 1965 samples and remained strong through 1968. The 1960 year-class was well represented in the 1966 through 1969 samples. In 1969 the newly recruited 1965 year-class was prominent in the samples.

The autumn fishery was characterized by the dominant 1958 year-class in 1965 samples. In 1966 through 1968 samples, the 1958 and 1959 year-

TABLE 1. Description of maturation stages of herring in the Gulf of St. Lawrence and the corresponding ICES herring maturity scale (Anon. 1962).

Maturation stage	Description	Corresponding ICES scale
Immature	Virgin or recovering herring. Gonads very small, eggs not visible to naked eye.	I, II, VIII
Maturing	Large gonads varying in size from less than half to almost as long as the body cavity. Eggs opaque, distinguishable by naked eye.	III, IV
Ripe and running	Gonads filling body cavity. Eggs large and transparent. Sperm and eggs flowing either freely or by slight pressure.	V, VI
Spent	Gonads baggy and bloodshot, sometimes containing remains of sperm or a few residual eggs.	VII

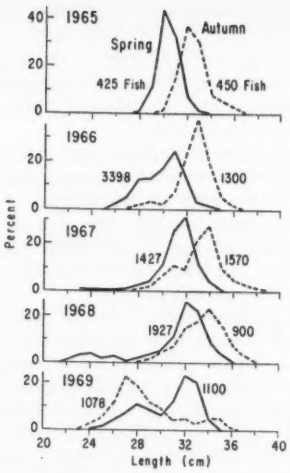

FIG. 3. Length-frequency distributions of herring from spring and autumn fisheries in the Gulf of St. Lawrence, 1965–69.

classes were strong. In 1969, the 1964 and 1965 year-classes made up nearly 55% of the samples.

When age-frequency distributions in the two fisheries for each year were subjected to a chi-square test, the values obtained were large ($P < 0.01$), rejecting the hypothesis that the samples were drawn from the same population.

Growth

When the back-calculated mean lengths were plotted according to Walford's method (Walford 1946), the points fell almost on straight lines. The plots were used to find trial values of the growth constant k, and these, in turn, were used to compute growth parameters and to plot growth curves (Fig. 5).

Growth curves for spring herring were nearly congruent and the maximum lengths were similar (Fig. 5). However, between spring and autumn herring, differences in growth curves and maximum lengths were obvious.

For autumn herring, maximum lengths were greater than spring herring, as were lengths at the end of their 1st year of life. The latter is more clearly shown from the l_1 frequency distributions for both groups (Fig. 6).

Maturation and Spawning

The percentage frequency distributions of maturation stages (Table 3) show that the majority

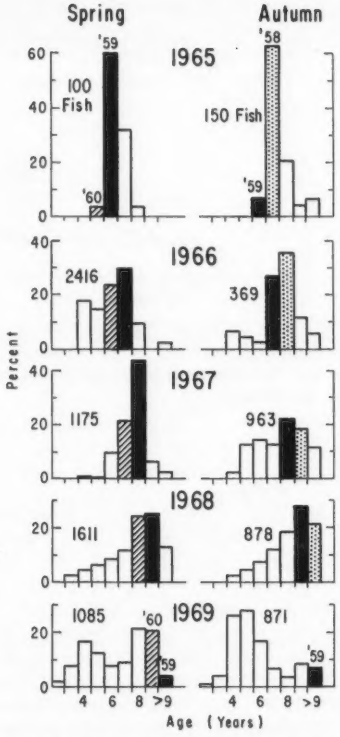

FIG. 4. Age-frequency distributions of herring from spring and autumn fisheries in the Gulf of St. Lawrence, 1965–69.

TABLE 2. Mean lengths and ages of herring in samples from spring and autumn fisheries in the Gulf of St. Lawrence, 1965–69.

	Spring fishery		Autumn fishery	
Year	No.	Length (*cm*)	No.	Length (*cm*)
1965	425	30.9±0.87[a]	450	33.1±1.25
1966	3398	29.9±2.67	1300	33.0±1.58
1967	1427	31.3±2.20	1570	33.4±2.02
1968	1927	30.3±4.15	900	32.9±2.39
1969	1100	30.2±3.13	1078	29.6±2.99
	No.	Age (*years*)	No.	Age (*years*)
1965	100	6.36±0.63	150	7.42±0.93
1966	2416	6.09±1.42	269	7.43±1.49
1967	1175	7.63±1.29	963	7.45±1.71
1968	1611	7.67±1.82	878	8.11±1.66
1969	1085	6.43±2.24	871	5.71±1.96

[a]One standard deviation.

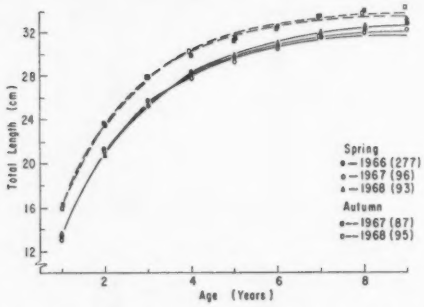

FIG. 5. Growth curves of herring from spring and autumn fisheries in the Gulf of St. Lawrence; curves fitted to von Bertalanffy growth equations. Numbers of fish in parentheses.

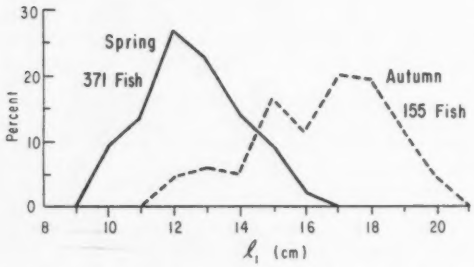

FIG. 6. Length-frequency distributions of herring at the end of first year of life (l_1) from spring and autumn fisheries in the Gulf of St. Lawrence, 1966–68.

TABLE 3. Percentage frequency distributions of maturation stages of herring from spring and autumn fisheries in the Gulf of St. Lawrence 1965–69; sexes combined.

Year	No. fish	Immature	Maturing	Ripe and running	Spent
		Spring fishery			
1965	100	–	–	85.0	15.0
1966	3197	5.0	3.8	86.1	5.1
1967	1178	1.5	7.8	85.8	4.8
1968	1850	9.4	24.0	65.8	0.8
1969	1096	11.1	8.7	77.7	2.4
		Autumn fishery			
1965	150	–	–	95.4	4.7
1966	400	2.3	2.0	95.5	0.3
1967	948	1.6	0.1	88.8	9.5
1968	809	7.3	24.7	61.2	6.8
1969	198	3.5	8.6	87.9	–

of fish in the samples were in the ripe and running stage.

If herring spawn only once each year, and all existing evidence supports this assumption, clearly the two fisheries exploit different stocks with different maturation cycles.

Spawning times for spring and autumn herring in the Gulf of St. Lawrence are strikingly regular. No data on the time of first arrival of spawners on the spawning grounds are available for the period of study, but data for an earlier period (1946–63) show that the times of first arrival varied little from year to year (Table 4).

For the present study, dates of peak spawnings in the Bay of Chaleur area were recorded and showed that over a 4-year peroid (1966–69) peak periods for spring spawners were from May 3 to 17 and those for autumn spawners from August 2 to 24. This shows rather conclusively that the spawning seasons of spring and autumn herring are relatively short and do not overlap.

Meristics

The meristic counts, taken only from 1969 samples (Table 5), showed that fin-ray counts were different for spring and autumn herring but that vertebral counts were similar. Autumn herring had, on the average, more rays in their dorsal

TABLE 4. Dates of first arrival of herring on spawning grounds in the Gulf of St. Lawrence 1946–63 (Lauzier and Tibbo 1965; Jean MS 1956) and of spawning peaks 1965–69 (present investigation).

Year	Locality	Spring fishery	Autumn fishery
1946	Magdalen Is.	Apr. 29	–
1948	"	30	–
1949	"	24	–
1950	"	30	–
1951	"	4	–
1952	Grand River, Bay of Chaleur	28	Aug. 14
1953	"	18	10
1954	"	23	16
1955	Magdalen Is.	18	–
1958	"	7	–
1960	"	8	–
1962	"	30	–
1963	"	29	–
1965	Caraquet, Bay of Chaleur	–	16
1966	"	May 17	2
1967	"	17	24
1968	"	6	17
1969	"	3	21

TABLE 5. Frequency distributions, means, and Z test between the means of meristic counts of herring from spring and autumn fisheries.

Fishery	Meristic counts					Total	Mean	Z
	Vertebra							
	53	54	55	56	57			
Spring	2	24	162	182	24	394	55.513±.725[a]	.784
Autumn	1	18	162	190	24	395	55.552±.690	
	Dorsal fin rays							
	17	18	19	20	21			
Spring	2	95	163	92	6	358	19.014±.783	2.727[b]
Autumn	5	59	201	109	12	386	19.166±.765	
	Pectoral fin rays							
16	17	18	19	20	21			
Spring 30	142	130	84	13	1	400	17.778±.985	5.690[b]
Autumn 10	63	153	116	12	–	354	18.161±.855	

[a]One standard deviation.
[b]Different at $P = 0.01$.

and pectoral fins, and for both, the differences are statistically significant.

Discussion

The evidence supports the hypothesis of discrete populations. In this study, length and age compositions, length at the end of the 1st year of life, growth, maturation cycles, spawning times, and fin-ray counts showed significant differences between spring- and autumn-herring populations in the southern Gulf of St. Lawrence. This is in contradiction to Jean's (MS 1956) hypothesis that the spawning populations in the spring and autumn fisheries are a mixture of fish that were hatched in the spring and autumn in similar proportions. However, his conclusion was based on the similarity in vertebral counts and head length/body length ratios — evidence that, we believe, is not sufficient to prove homogeneity. Moreover, Jean (MS 1956) assigned fish to a hatching season on the basis of "scale ratios." This is a phenotypic character of questionable validity, since there was a substantial amount of overlapping between the scale types. Messieh (1969) suggested that variable hydrographic conditions might affect otolith patterns of herring from a single spawning, and it is quite possible that scale patterns are affected in a similar way.

Tibbo (1957) reported similarities in mean vertebral counts of spring- and autumn-spawning populations, although he stated "there is little doubt that there are at least two fairly discrete groups of herring, that are fished from Bonaventure and Caraquet, respectively." Day (1957) considered the possibility of separate populations on the basis of differences in growth and maturity stages but wondered why fall-spawning herring differed from spring spawners in growth, yet did not differ significantly in vertebral counts. The lack of significant differences in vertebral count was confirmed in the present study. Similarly, Parsons and Hodder (1971) and Hodder and Parsons (1971) found similar vertebral counts between spring- and autumn-spawning herring off the southwest coast of Newfoundland.

Although data in the present study do not allow examination of the genetic isolation of spring and autumn spawners, the year-classes recruited to a given spawning ground appear to return to the same ground and fishery year after year. This is the most logical explanation for the similarities in growth curves in the two fisheries, the shift in modal lengths from year to year, and the differences in maturation cycles. Cushing (1967) suggested that differences in spawning times are linked to production cycles and, if fish spawned in a fixed season, the population has the best chance of profiting by the variability of the production cycle. It should be emphasized, however, that conclusive evidence

on the biological isolation of the two populations will require demonstration of genetic differences.

For fisheries management purposes, however, it is sufficient to demonstrate that each of the herring populations in the spring and autumn fisheries in the Gulf of St. Lawrence is relatively homogeneous and self-contained, i.e., a separate stock.

Acknowledgments

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Lysosomal Triglyceride Lipase from the Lateral Line Tissue of Rainbow Trout (*Salmo gairdneri*)[1]

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Bilinski, E., R. E. E. Jonas, and Y. C. Lau. 1971. Lysosomal triglyceride lipase from the lateral line tissue of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Bd. Canada 28: 1015–1018.

An acid lipase active toward tripalmitin and having the characteristics of lysosomal enzymes was shown to occur in the red lateral line tissue of rainbow trout (*Salmo gairdneri*). The enzyme showed maximum activity at *p*H 4–4.5. Triton X-100 (0.2–2.0%) strongly stimulated the activity of the acid lipase, but it inhibited markedly the lipolytic activity above *p*H 7. NaF (20 mM) and Na-*p*-chloromercuriphenyl-sulfonate (1 mM) partially inhibited the acid lipase. Fractionation of the total homogenate by differential centrifugation in 0.25 M sucrose showed that the acid lipase was present at highest concentration in the light mitochondrial fraction. Palmitic acid and dipalmitin were the two main products of hydrolysis of tripalmitin.

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The occurrence of lysosomes, as distinctive subcellular organelles containing a number of acid hydrolytic enzymes that are responsible for the catabolic processes of cells, is now well established (De Duve and Wattiaux 1966). In fish, in a study conducted on lysosomal enzymes in the white muscle of goldfish, Bird et al. (1969) presented evidence for the presence of cathepsin D, acid ribonuclease, aryl sulfatase, and β-glucuronidase. It does not appear that the hydrolysis of triglycerides by lysosomal enzymes was demonstrated in muscle, but it was shown to take place in other tissues in mammals (Hayase and Tappel 1970 and references cited; Fowler and De Duve 1969; Guder et al. 1969) and in toad bladder epithelium (Rosenbloom and Elsbach 1969). The present report provides evidence for the occurrence in the red lateral line tissue of rainbow trout, of an acid triglyceride lipase having the properties of lysosomal enzymes.

Materials and Methods

Fish

Rainbow trout (*Salmo gairdneri*) were obtained from Sun Valley Trout Farm, British Columbia. They were of both sexes and had an average weight of 250 g. Fish were maintained under natural photoperiod in a freshwater aquarium at 12–15 C. They were fed a mixture of 80 parts beef liver, 80 parts beef heart, 80 parts canned salmon, 4 parts pablum, and 1 part sodium chloride. The fish used had not been fed for about 20 hr prior to being killed.

Tissue Preparation

The lateral line tissue containing the red muscle was used as starting material for tissue preparations. Besides muscle fibers, this tissue contains also other types of cells (connective tissue, fat, etc.). It was, however, not feasible to isolate pure muscle for this study.

The following procedure was used for the preparation of homogenates. The red lateral line tissue was isolated by the method described previously (Bilinski and Lau 1969). A portion of the tissue (usually 350 mg), cut into small pieces with scissors, was placed in a test tube (18 × 150 mm) containing 2.0 ml of buffer solution, and maintained in an ice bath. The tissue was disrupted by 30 gentle up-and-down strokes made with a motor-driven Teflon pestle rotating about 1400 rpm. The other constitutents of the incubation mixture, including Triton X-100, were added only after homogenization.

For fractionation of the subcellular constitutents, the total homogenate was obtained by the procedure described above, by pooling the tissue homogenized in test tubes with 0.25 M sucrose. For differential centrifugation, the scheme of De Duve et al. (1955), as described by Bird et al. (1969) in a study of fish muscle lysosomes, has been used. The various subcellular fractions listed in Table 2 were obtained under the following centrifugal conditions: heavy mitochondria — 10,000 *g* for 5 min: light mitochondria — 22,500 *g* for 10 min; microsomes — 75,000 *g* for 45 min.

[1]The seventh paper in a series on utilization of lipids by fish.

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Nitrogen was determined by the Kjeldahl method (Steyermark 1951).

Determination of Lipase Activity

Incubation was conducted at 15 C in a refrigerated constant temperature bath provided with a shaking device. The incubation mixture (total volume 4 ml) had the composition indicated in the tables and figures. Glyceryl tri (palmitate-1-^{14}C) was obtained from New England Nuclear Corp., Boston, Massachusetts, and before use as substrate it was emulsified with phospholipid (1 mg phospholipid per 50 mμmoles tripalmitin), as previously described (Bilinski and Lau 1969). At the end of the incubation a total lipid extract in *n*-heptane was prepared as indicated earlier (Bilinski and Lau 1969) by the procedure of Carter (1967). When the lipolytic activity was measured solely by the release of palmitic acid, the ^{14}C palmitic acid was separated from the *n*-heptane extract by the alcoholic NaOH partition method of Borgström (1952) and its radioactivity was determined by scintillation counting (Bjornstad 1966). When hydrolysis of tripalmitin to mono- and di-palmitin was also determined, neutral lipids were first isolated from the *n*-heptane extract by elution with chloroform from silicic acid (5 g silicic acid; 250 ml chloroform) and then separated by thin-layer chromatography on Eastman Chromatogram Silica Gel Sheets, Type K 301R2, with a solvent system composed of petroleum ether (bp 60–110 C)–diethyl ether–glacial acetic acid, 90:10:1, v/v (Malin and Mangold 1960). The various compounds were identified on thin-layer sheets as previously described (Bilinski and Lau 1969).

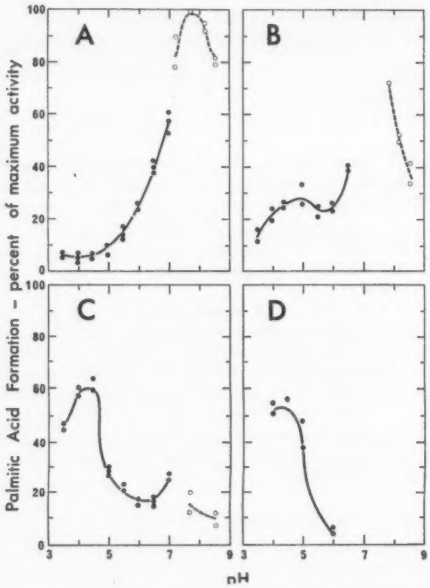

FIG. 1. Effects of *p*H and Triton X-100 on lipase activity: A, without Triton X-100; B, 0.2% Triton X-100; C, 2.0% Triton X-100; D, 8.0% Triton X-100. ●, Na citrate–phosphate buffer, 100 mM (*p*H 3.5–7.0); ○, Tris, 100 mM (*p*H 7.2–8.6). Incubation for 60 min with 40 mμmoles ^{14}C-tripalmitin and 350 mg lateral line tissue homogenate.

Results

The effects of *p*H and of the addition of Triton X-100 on the hydrolysis of tripalmitin to palmitic acid were determined using homogenates of the red lateral line tissue. In absence of Triton X-100 (Fig. 1A) the maximum activity was above *p*H 7.5 and lowering of *p*H in the acid range resulted in a sharp decrease in activity. At *p*H 3.5–5.0 only a very limited activity was observed. The effect of Triton X-100 on activity was dependent on *p*H. Concentrations of 0.2% Triton X-100 activated lipolysis in the acid and inhibited in the alkaline range. This trend was more pronounced at the higher Triton X-100 concentration. In presence of 2% Triton X-100 (Fig. 1C) the highest lipolytic activity was observed at *p*H 4–4.5, whereas the activity at *p*H 7.5 was 80% inhibited. The increase of Triton X-100 concentration to 8% (Fig. 1D) did not result in a further increase in lipolytic activity. In subsequent experiments certain properties of the acid lipase were determined at *p*H 4.5 in presence of 2% Triton X-100. Under these conditions the formation of palmitic acid from tripalmitin was proportional to the amount of tissue, and it was linear with respect to time during a 2-hr incubation period at 15 C (Fig. 2). The enzyme was partially inhibited by NaF (20–200 mM) and by the -SH group reagent, *p*-chloromercuriphenyl sulfonate (Table 1), but EDTA (1 mM) and protamine sulfate (0.3 mg/ml) had no inhibitory effect. Fractionation of the total homogenate by differential centrifugation in 0.25 M sucrose showed that the acid lipase was present at highest concentration in the light mitochondrial fraction, which has the sedimentation characteristics of lysosome, but at a lower level of activity the enzyme occurred also in other subcellular constituents (Table 2). Determination of the products released by the action of the trout acid lipase on tripalmitin was made by thin-layer chromatography. Palmitic acid and dipalmitin were found to accumulate during hydrolysis (Table 3). Using thin-layer chromatography it was also confirmed that under the conditions used, the ethanol NaOH partition method of Borgström (1952) is specific for the determination of radioactivity in palmitic acid.

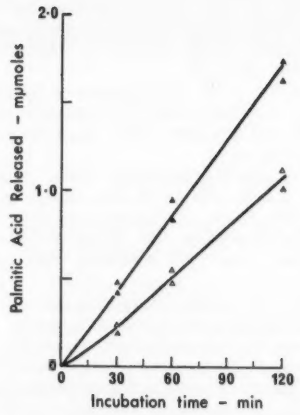

FIG. 2. Effects of incubation time and amount of tissue on acid lipase activity. 200 mg (△) and 350 mg (▲) of lateral line tissue homogenate. Other incubation conditions the same as in Table 1.

TABLE 1. Inhibition of acid lipase. Incubation conditions: 120 min at 15 C; Na citrate–phosphate, *p*H 4.5, 100 mM; Triton X-100, 2%; ^{14}C-tripalmitin, 40 mμmoles; lateral line tissue homogenate, 350 mg.

Compound added	Relative activity
Control	100
NaF, 20 mM	34, 36
NaF, 200 mM	22, 24
Na-*p*-chloromercuriphenyl-sulfonate, 1 mM	34, 37

TABLE 2. Acid lipase activity in subcellular fractions of lateral line tissue.

Subcellular fraction[a]	Specific activity[b] Expt 1	Expt 2
Heavy mitochondria	2.04	4.32
	2.52	4.02
Light mitochondria	6.61	11.35
	6.51	11.92
Microsomes	2.19	2.62
	2.97	2.44
Postmicrosomal supernate	1.30	1.50
	1.19	1.47

[a]For the centrifugal fractionation procedure, see Material and Methods.

[b]The specific activity, i.e., activity per milligram protein N, is expressed in terms of that of total homogenate, which is taken as equal to 1. Incubation conditions: 60 min at 15 C; Na citrate–phosphate, *p*H 4.5, 100 mM; Triton X-100, 2%; sucrose, 500 mM ^{14}C-tripalmitin, 40 mμmoles.

TABLE 3. Determination of the products of tripalmitin hydrolysis by the acid lipase.[a]

Expt	% on molar basis Palmitic acid	Monopalmitin	Di-palmitin
1	31.4	24.6	44.0
2	49.4	2.7	47.9
3	37.7	7.2	55.1

[a]For the thin-layer chromatography procedure, see Material and Methods. Incubation conditions, the same as in Table 1.

Discussion

Our results indicate the presence in the trout lateral line tissue of an acid long-chain triglyceride lipase, having the characteristics of lysosomal enzymes. This enzyme is different from the triglyceride lipase(s), previously demonstrated in this tissue, which has been shown to have a maximum activity above *p*H 7 (Bilinski and Lau 1969). The distinctive nature of these two lipases is also supported by the fact that the nonionic detergent Triton X-100 strongly stimulated the activity of acid lipase and markedly inhibited the lipolytic activity above *p*H. The maximum activity at *p*H 4–4.5, shown by the acid lipase, falls within the optimum *p*H range exhibited by the lysosomal acid hydrolases (De Duve and Wattiaux 1966). Our data on intracellular occurrence of the acid lipase indicate also that it is found at highest concentration in the lysosome-rich mitochondrial fraction. The lysosomal nature of trout lipase is also suggested by the latency of the enzyme. Enzyme latency, which may be suppressed by treatment with Triton X-100, has been observed with all lysosomal enzymes (Wattiaux and De Duve 1956). Treatment with 0.2% Triton X-100 was found sufficient to cause a complete loss of latency of the various enzymes studied in the white muscle of goldfish by Bird et al. (1969), whereas in the case of acid lipase, the Triton X-100 concentration had to be raised to 2% to obtain this effect. Triton X-100 at comparably high concentration was shown by Mahadevan and Tappel (1968) to be required for the maximum activity of rat liver lysosomal lipase, and Fowler and De Duve (1969) have also found that tripalmitin is resistant to hydrolysis

by rat lysosomes in absence of Triton X-100. On the other hand, Triton X-100 was shown to inhibit the rat adipose tissue triglyceride lipase, active at *p*H 7.2 (Gorin and Shafrir 1964). By leading to formation of free fatty acids and diglycerides, as the two main products of hydrolysis of triglycerides, trout acid lipase shows similarities with the rat liver lysosomal lipase (Hayase and Tappel 1970).

The physiological function of lysosomes is thought to consist of intracellular digestion of cell constituents, which may take place under normal physiological conditions or have the character of pathological response in cells subjected to some kind of metabolic stress (for review see De Duve and Wattiaux 1966; Dingle and Fell 1969). The occurrence of lysosomal lipase in the red lateral line tissue of trout is of interest, since this type of muscular tissue of fish is particularly rich in fat, and it has been shown to be actively involved in the catabolic reactions of lipids (Bilinski 1963; Bilinski and Jonas 1970; George 1962; George and Bokdawala 1964; Bokdawala and George 1967). With reference to salmonid fish, one may consider that in spawning salmon prior to its death, when tissues undergo extensive degradation, the lysosomal lipase may play a prominent function in the breakdown of muscle triglycerides. As pointed out earlier by Bird et al. (1969), the study of lysosomal enzymes in fish muscle may be of significance in understanding postmortem changes, where the muscle has a *p*H range of 5.5–6.6 (Buttkus and Tomlinson 1966). In this respect, the lipolytic enzymes may be of particular importance, since lipid hydrolysis during storage of fish has been related to the deterioration of the quality of fish.

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Role of Social Behavior in Dispersal of Introduced Rainbow Trout (*Salmo gairdneri*)

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JENKINS, T. M., JR. 1971. Role of social behavior in dispersal of introduced rainbow trout (*Salmo gairdneri*). J. Fish. Res. Bd. Canada 28: 1019–1027.

Hatchery-reared rainbow trout (*Salmo gairdneri*) planted under partially controlled conditions in an observation stream failed to show social density regulation or socially induced dispersal. Aggression frequency was only an imprecise function of density, and density may not have been influenced by aggression at all. Dispersal following planting was extensive, but it apparently resulted from individual differences in response to the habitat. Group behavior (schooling) affected dispersal radically in some instances, when fish in large groups undertook movements they would not have undertaken alone or in small groups.

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ONE of the critical assumptions in recent discussion of salmonid population dynamics is that social behavior regulates the density of fish in flowing water habitats (e.g., McFadden 1969). The actual mode of elimination of excess individuals is generally presumed to be through forced emigration. Chapman (1966) and Chapman and Bjornn (1969) have elaborated this viewpoint in considerable detail, and Chapman (1962) demonstrated the phenomenon fairly conclusively in juveniles of an anadromous species (*Oncorhynchus kisutch*). If the concept is applicable to stream salmonids in general, social interactions would presumably equalize density in spatially unbalanced resident stream populations. One way in which such populations are commonly produced is by artificial introduction of hatchery-reared fish to empty or near-empty streams. Planted fish are known to disperse to a highly variable degree, but the extent to which social behavior motivates this dispersal is unknown. One object of this study was to describe quantitatively the dispersal of various-sized groups of trout planted under partially controlled conditions, and to assess from these data whether social density control can be postulated. A second objective was to analyze the relation between agonistic behavior and dispersal to determine the mechanism of social density equalization (should it occur). The simplest such mechanism in the social context of the study stream (Jenkins 1969) would be a negative feedback loop involving positive effects of density on aggression and negative effects of aggression on density. I therefore looked in detail at the relation between group aggression frequency and density, and at the effectiveness of aggression in reducing density.

Hatchery-reared rainbow trout (*Salmo gairdneri*) were used for this study because of their availability and relative uniformity of previous experience, and because they closely resemble wild fish in stream social behavior and general ecology (Jenkins 1969).

Materials and Methods

THE STREAM

The study stream was a small channel supplied with water from Convict Creek, in the Sierra Nevada range of eastern California. Although the general channel configuration was artificial, the biological and physical details were those of a natural stream (see Jenkins 1969). The physical feature important for this study was nearly constant discharge of about 0.06 m³/sec, associated with a stable channel configuration. The principal biological features included the absence of extraneous fish and a supply of natural stream food, which frequent drift sampling and stomach content analysis have shown to be fairly uniform in characteristics and abundance through the summer months of July to September (Jenkins unpublished data). These features presumably provided a stream with relatively constant trout-habitat qualities. Water temperatures and weather conditions for all research days were recorded, but were found not to be correlated with fish behavior, and the details are not given here.

The experimental apparatus was a stretch of stream approximately 137 m in length, bounded at the upstream end by a simple funnel trap and on the downstream end by a modified Wolf trap (Fig. 1). The central 60 m were divided into four segments by means of concrete structures housing mechanically operated retaining screens,

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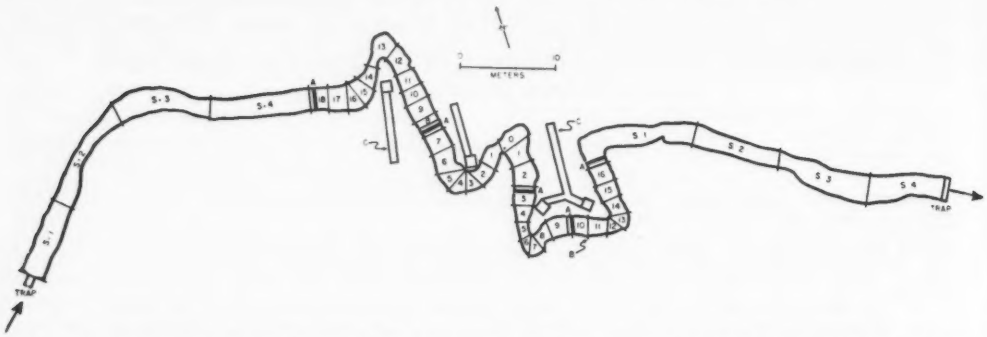

FIG. 1. The experimental stream, showing the locations of intervals discussed in the text. The point of introduction in zero interval is marked by an ×. A's designate the locations of mechanical barriers; B indicates a twine component of the grid; C's indicate two of the three observation tower systems.

with which groups were confined and released without being disturbed. Each segment was overlooked by a 3–5-m tall observation tower, enclosed but for observation windows and provided with tunnels for concealing an entering observer. Any path between towers where the observer could be seen by the fish was adequately shielded.

A one-dimensional grid was installed to record the linear positions of individuals, consisting of stakes delimiting 2- or 10-m intervals along one bank of the stream, and twine extending across the stream to other stakes (see Fig. 1). The pool at interval zero was near the center of the test stream, so all fish were introduced there. Judging from the fact that this interval attracts most of the fish immigrating from Convict Creek, it is probably the most desirable area in the stream.

THE FISH

The fish studied were underyearling (9–12 months old) rainbow trout of the Hot Creek fall-spawning strain and yearling rainbow trout of the spring-spawning Whitney strain. Two groups of 15 wild, adult brown trout (*Salmo trutta*) from Convict Creek were studied for comparison with the rainbow trout. All fish were held until used in 200 × 56 × 28.5-cm deep rectangular holding tanks, and fed on a maintenance diet of pelleted trout food. Water drawn from the source of the test stream was supplied to each tank at about 10 liters/min.

All fish were distinctively heat-marked (Jenkins and Klain 1969) from a day to as long as a month before they were used. Experimental groups were drawn from one tank until about 15 fish remained. Those were then added to the tank from which the next group was to be taken. Experimental groups were composed of individuals selected as randomly as possible from the holding tanks, at a time no more than 10 min before their introduction to the stream.

EXPERIMENTAL PROCEDURES

Two general types of experiment were carried out, according to a schedule designed to spread replications as much in time as possible (Table 1). These were the *open-stream* and *initial-confinement* experiments.

In open-stream experiments, groups of 5, 15, and 25 fish, or single individuals were planted in the stream at 6:00 AM (Pacific Standard Time) at the zero interval. I immediately entered the nearest tower and observed behavior intermittently for 4 hr, or until no fish remained visible. At 10:00 AM, 2:00 PM, and 6:00 PM on the day of introduction, and at 4-hr intervals from 6:00 AM to 6:00 PM on the following days, I checked the traps, observed the stream, and identified and noted the positions of all visible fish. More lengthy observations were made if fish were moving or fighting. Trapped fish were weighed and measured. All fish were removed by electrofishing and measured after the 6:00 PM observation on the 5th day, except in instances where no more than one fish remained in the zero interval and no more than two remained in the area visible from towers. Fish were removed immediately if observation indicated such a circumstance.

In initial-confinement experiments, planting procedure was identical to that in open stream experiments, but screens upstream and downstream from the point of introduction were temporarily closed. By this means I sought to separate effects of entering an unfamiliar stream from effects of social interaction. In 1968 trials, fish were confined in intervals –3 to +7 for 4 full days. Positions and general behavior (agonistic acts and individual positions in particular) were observed from 6:00 AM to 6:00 PM on the first day, and from noon to 6:00 PM on the following 3 days. At 6:00 AM on the 5th day, the screens were gently raised and procedures of the open stream experiments begun. The open stream phase was terminated on the 9th day, unless early termination was called for (see above). In 1970 initial-confinement experiments, fish were confined for only 24 hr, and were free to move for at least 5 days after the screens were raised. Observations of position choice were made every 4 hr from 6:00 AM to 6:00 PM on the first 6 days of these trials. If the experiments were extended beyond 6 days (experiments 39–41), observations were made sporadically on subsequent days.

TABLE. 1. Schedule of operations for 41 experiments during the summers of 1968–70. In 1968, experiments 2, 3, 8, 9, and 13 were added to study the relation of group size to schooling and mass migration. Experiments 8 and 9 involved brown trout and the rest rainbow trout. Experiments 39–41 used Whitney strain fish, and the others Hot Creek Strain fish.

Expt no.	Type of expt	Began	Ended	Group size
		1968		
1	Open stream	July 3	July 3	15
2	"	4	4	15
3	"	5	6	15
4	"	7	8	5
5	"	9	9	25
6	Confined	11	19	15
7	Confined	22	30	25
8	Open stream	31	31	15
9	Open stream	Aug. 1	Aug. 1	15
10	Confined	2	7	5
11	Open stream	8	12	15
12	"	13	17	25
13	"	18	19	15
14	Confined	20	28	25
15	"	31	Sept. 8	15
16	"	Sept. 9	17	5
17	Open stream	18	19	5
		1969		
18	"	June 18	June 18	1
19	"	19	20	1
20	"	21	21	1
21	"	22	22	1
22	"	23	24	1
23	"	24	24	1
24	"	25	25	1
25	"	26	26	5
26	"	27	28	1
27	"	28	28	1
28	"	29	29	1
29	"	30	July 1	5
30	"	July 1	2	10
		1970		
31	Confined	June 21	June 26	25
32	"	27	July 2	100
33	"	July 3	8	75
34	"	9	14	50
35	"	15	20	25
36	"	21	26	100
37	"	15	20	75
38	"	Aug. 3	Aug. 8	50
39	"	12	19	25
40	"	19	Sept. 3	25
41	"	Sept. 3	19	43

Agonistic acts were recorded on a high speed 10-channel event recorder and keyboard, with the observer in an exceptionally good vantage point. I had no difficulty recognizing and tabulating overt acts (involving fin position or locomotion) even in the densest groups of trout. However, when observing groups composed of more than five fish, I was unable to determine the individuals involved in each bout.

In experiments 14–16, all aggressive acts taking place in intervals –3 to +2 on days 3, 4, 6, and 9, between the hours of noon and 4:00 PM were recorded. In experiments 32 and 34–38, aggressive acts in the same area during the hours from 10:00 AM to 2:00 PM on the 3rd and 6th days were recorded. In experiments 40 and 41, aggressive acts in this area from 10:00 AM to 2:00 PM on the final days only were recorded.

The area included in intervals –3 to +2 was the primary spatial unit considered in this study, and will hereafter be termed the *area of introduction*. It consists of a small pool (where the fish were introduced) bounded by shallow riffle on either end, including about 15 m of channel and 22 m² of water surface. This unit has been shown (Jenkins 1969) to be nonarbitrary, in the sense that fish holding positions there always flee to interval zero when disturbed, and prefer holding positions in that interval. They distribute within the unit as a consequence of agonistic behavior. Such groups with common primary position preferences form the largest population unit in this stream wherein all members interact with significant frequency.

Results

Initial Behavior

The experimental trout behaved in a consistent manner upon entering the stream. They first congregated in the deepest area of the zero interval pool; then most individuals joined to form one or more schools. Occasionally a fish left the area entirely before schools were fully formed, or remained nearby and declined to join. The schools forayed to gradually increasing distances from the initial point of congregation, and, if not confined, left the area entirely (migrated) or broke into fighting pairs. School breakdown was followed by dispersal rather than migration (see below).

What I have defined as migration and dispersal were quite distinctive with respect to movement timing and associated behavior. *Migration* was rapid movement by a school after it left the area of introduction. Within minutes all fish involved reached the vicinity of a terminal trap. Once at the trap, most entered without hesitation, but occasionally an individual waited for an hour or two. Migration involved social behavior only in the broad sense of school interactions. *Dispersal* occurred in experiments where schools broke down before leaving the vicinity of introduction. It involved individual movements at various speeds

to varied distances. Although most dispersal was in the downstream direction, it was not universally downstream like migration in rainbow trout groups (Fig. 2). Because onset of agonistic behavior was simultaneous with (and perhaps responsible for) school breakdown, dispersal was temporally related to agonistic interactions. However, agonistic interactions always persisted after dispersal ceased.

Migration immediately after planting took place only in the groups of 15 and 25 fish in early summer, 1968 (experiments 1–3, 5, 8, and 9), but confined groups of 15 and 25 fish planted during the same period (experiments 6 and 7) tried to migrate as well. When the latter were finally released after 4 days, they dispersed instead of migrating. Schools in these groups actually broke into fighting pairs within a few hours of planting. Even *unconfined* five-fish groups dispersed rather than migrating, because their schools broke down shortly after forming.

Most importantly, these observations indicate that the movement behavior of fish was affected by the size of group they were in. Fish in the large (15 and 25 fish) migrating groups reached their final positions (usually terminal traps) much earlier than fish in comparable small (five fish) groups, and their final positions always averaged further from the point of introduction. Since migrating fish traveled faster than dispersing fish, they might also have moved greater distances had the stream been longer.

The large groups in 1970 experiments were temporarily confined to eliminate mass movements, but group behavior was not entirely absent at the time of release. A few fish dispersed within the area of confinement, whereas the majority remained in tight zero-interval aggregations until screens were raised. Judging from the fact that some fish left the area of initial confinement in small groups, and several often entered the downstream trap, simultaneously, there was probably some group enhancement of individual movements like that mentioned above.

Timing of Dispersal

Rate of dispersal was always highest during the first 4 hr after release, and declined steadily until about the 5th day (Fig. 3). After that, nearly all drops in number in the area of introduction were temporary, due to short-range movements of some peripheral fish. Fish in experiments 40 and 41, which coincidentally left the highest densities in the area of introduction, were allowed to remain in the stream for 16 or 17 days rather than 5 or 6 as in the other experiments. Yet there was no significant dispersal after the 2nd day.

Evidence for Nonsocial Dispersal

Had dispersal been entirely a manifestation of density regulation, none should have occurred until plantings reached a certain size. Above that size, the percentage emigrating from the area of introduction should have increased progressively. Dispersal did not follow this pattern, and there was no indication that it was socially motivated in smaller groups. None of the 10 isolated fish (experiments 18–24, 26–28) chose to remain where planted, and they in fact moved on the average as fast and as far as fish in groups. For each fish in isolate and group experiments of 1969 an index of dispersal was calculated, accounting for timing and distance of movement. Approximate distance from the point of introduction was plotted against time, and area under the curve integrated to provide an index of time–distance units. Distributions of the magnitude of this index were similar for isolates and fish in groups of five and 10 (Fig. 4), suggesting that presence or absence of conspecifics was of little importance to a fish's movements.

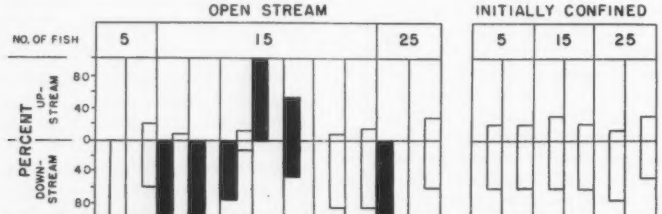

Fig. 2. Percent of fish in the 17 experimental groups in 1968 that had moved upstream or downstream from the area of introduction by experiment termination. "No. of fish" refers to original group size. Fish remaining in the area of introduction are not included. ■, migrate; □, disperse.

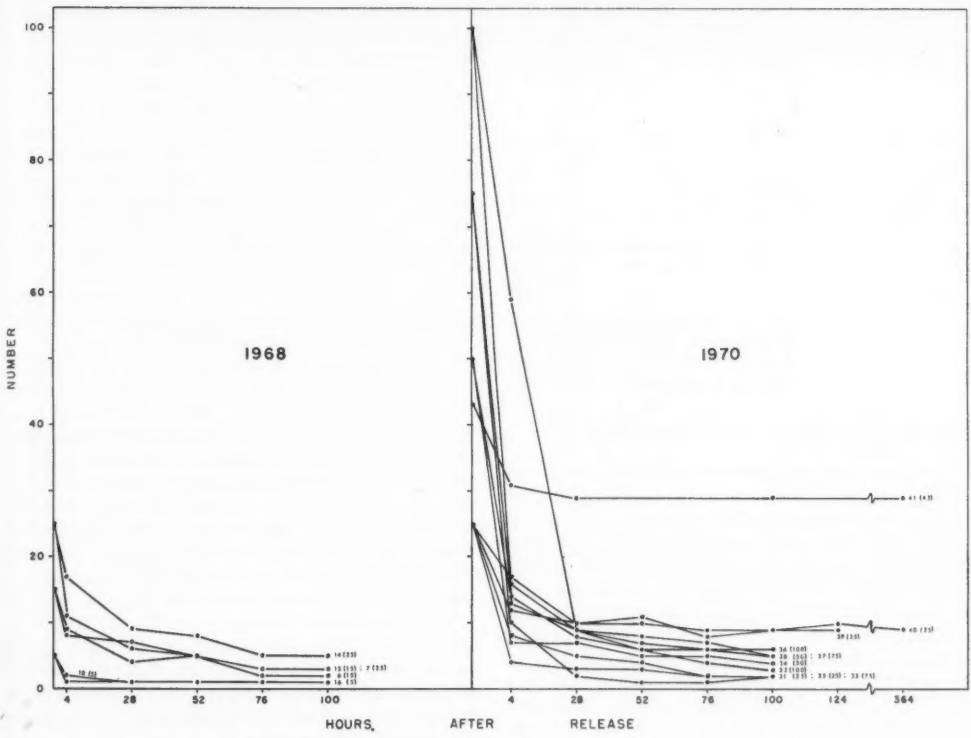

FIG. 3. Numbers of fish in the area of introduction (intervals −3 to +2) in relation to time after planting, summers 1968 and 1970. All groups were initially confined, and released at time zero (see Table 1). The curve for each group is labeled with experiment number and initial group size (in parentheses). Numbers of fish are from the 10:00 AM observations. Note that the scale for 1970 experiments has been tripled for the first 4 hr to show more detail.

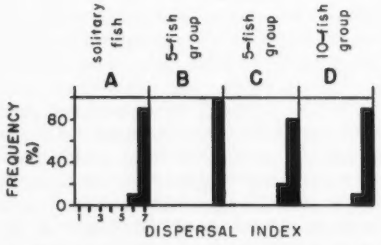

FIG. 4. Frequency-percent histograms showing the proportion of fish in each 1969 experiment (or series of experiments) whose mobility indices fell within the ranges numbered on the horizontal axis. Each range is equivalent to 20 mobility units, except for the last, which is equivalent to 12.5. Histogram A combines the data for all 10 solitary fish (experiments 18–24, 26–28). Histograms B and C deal with groups of 5 fish (experiments 25, 29), and D with a 10-fish group (experiment 30).

Agonistic behavior was not, in fact, closely related to movements in any of the 1968 groups. I found that movements of fish in groups of 5, which could be studied in greatest detail, were not detectably related to their social situations or the types of agonistic behavior going on about them. More specifically, neither the status of fish that were members of social hierarchies at the time of release (initial-confinement experiments 10, 16), nor the frequency, intensity, or outcome of bouts in which fish participated when dispersal and hierarchy establishment coincided (open-stream experiments 4, 17) were related to the direction, speed, timing, or ultimate extent of their movements (narratives available on request). The same was true for fish that I was able to follow in 15- and 25-fish groups. It is also significant that fish size and dispersal index were unrelated (Fig. 5), although social status is based primarily on size in these fish (Jenkins 1969).

FIG. 5. Scatter plot of dispersal index against standard length of fish in dispersing open stream and initially confined groups of rainbow trout, summer 1968. Fish in experiments in which mass migration occurred are omitted (experiments 1–3 and 5).

RELATION OF INITIAL TO FINAL DENSITIES

Provided certain sources of variation are accounted for, the final densities from dispersing groups in 1968 were approximately proportional to their initial sizes. Initial confinement for 4 days increased final density, and groups planted late in the summer dispersed less than those planted earlier, so only fish at the same time of summer and undergoing the same treatment can be compared. Early-summer initially confined groups of 5, 15, and 25 left 20,[1] 13, and 12% of their number in the area of introduction. Late-summer initially confined groups of 5, 15, and 25 all left 20% in the area of introduction. Late-summer unconfined groups of 5, 15, and 25 left 20,[1] 7, and 8% in the same area. (Early summer unconfined groups all migrated, leaving none in the area of introduction.)

Even if the remnants of 25-fish groups are too small because of nonsocial dispersal to elicit socially induced dispersal, one should be able to surpass social "carrying capacity" in a given area by planting progressively larger groups. Eventually we should see socially induced dispersal superimposed on nonsocial dispersal. The 1970 experiments, with initial group sizes up to 100 fish, were directed toward this end.

Differences between densities left in the area of introduction by 25- and 50-fish Hot Creek groups were again proportional to their initial sizes, but densities were still low. Two groups of 25 both left 8% in the area of introduction, and two groups of 50 left 8 and 10%. Plantings of 75 or 100 fish failed to increase density significantly, suggesting that compensatory dispersal was taking place. Two groups of 75 fish left 3 and 7%, and two groups of 100 left 3 and 6%. However, most dispersal in the largest groups occurred during the first 4 hr, or during the first night. Group behavior during these periods (see initial behavior section) could have increased movement enough to give a false impression of density regulation, especially since 4 or 1 fewer emigrants from the two 75-fish groups and 5 or 2 fewer from the two 100-fish groups would have given them the same *percentage* emigration from the area of introduction as in the 25- and 50-fish groups.

It is also important that fish emigrating during all phases of 1970 experiments were just as large as fish remaining where planted. Fish leaving the area of introduction during the first 2 days were virtually random samples of the original populations. For the slow-dispersal phases of the experiments (days 3–6), I compared the standard lengths of all fish remaining in the area of introduction at experiment end with those that left after 10:00 AM on day 3. There were no significant size differences between the residual and emigrating fish at the 5% probability level, within any individual experiments (randomization test, Siegel 1956), or between pooled samples comparing all emigrants with all stable fish (*t*-test, 58 df). As mentioned earlier, size generally determines success in aggression in hatchery trout, so it would appear that emigration was not forced by social subordination.

Whitney-strain fish dispersed less than the Hot Creek fish, particularly during the first 4 hr after release (see Fig. 3). Whitney fish averaged 37.3% loss from intervals −3 to +2 during the first 4 hr, as compared with 75.6% for 1970 Hot Creek fish

[1]Twenty percent, or one fish, is possibly an overestimate, because these experiments were terminated early (see Materials and Methods). These individuals might have left the area of introduction had they remained for the full 5 days.

($P < .005$, t-test, 9 df). There was no indication that the two strains of rainbow trout differed behaviorally, other than in their tendency to disperse. I presume the dispersal that did occur in Whitney groups was nonsocial in motivation, since its timing was similar to that in small Hot Creek groups, and the proportion remaining in the area of introduction actually increased with increasing initial group size. Thirty-six percent of both groups of 25 but 67% of the group of 43 fish remained in the area of introduction. Since nonsocial factors such as time of summer and initial confinement seem to have influenced dispersal in earlier experiments, it is probable that some such factor decreased dispersal in the 43-fish group relative to the others. In any case, data for the Whitney strain are relevant to interpretation of the Hot Creek results. Unless the strains were quite different in some subtle respect, it is difficult to see why social interactions would force fish from groups of 5 or more of one strain (Hot Creek) but not from groups of 9 or 29 of another. I therefore conclude that social density limitation through forced emigration from areas of high density was inoperative or ineffectual in both strains of trout.

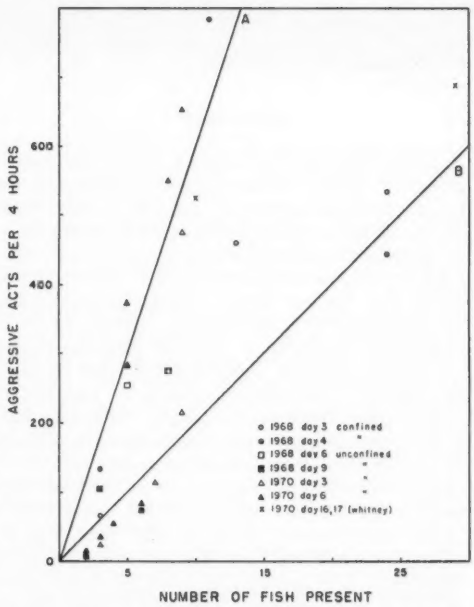

FIG. 6. Numbers of aggressive acts occurring in the area of introduction (intervals −3 to +2) in relation to number of fish present, summers 1968 and 1970. Line A is equivalent to 60 aggressive acts/4 hr per fish, and line B is equivalent to 20 acts/4 hr per fish.

RELATION OF AGONISTIC BEHAVIOR TO DENSITY

The effect of density on aggression was quite complicated in these experiments, because agonistic behavior in recently formed groups changes with time (Jenkins 1969). Therefore, only groups at the same stage in formation of their social structures are strictly comparable. Such a comparison (Fig. 6) shows that aggression tends to be more frequent when more fish are present, but that frequency of aggression in different groups of the same size can vary tremendously. For example, three 1970 Hot Creek groups left 9 fish in the area of introduction on the 3rd day, but they produced aggression frequencies from 218 to 659 acts per 4 hr. Similarly, a group of 24 interacting fish (1968) produced fewer aggressions than 12 fish in a comparable group. Thus aggression frequency is to only a limited extent a predictable consequence of density, and a fish might see — or be involved in — more agonistic bouts in sparse groups than dense groups.

Variability in frequency of aggression at a given density might in itself have accounted for the inaccuracy of density regulation, had aggression frequency always been the same when groups ceased declining in size. This was not the case. Aggression frequency in Hot Creek groups of 5 or larger varied from 83 to 375 per 4 hr on the 6th day, by which time dispersal had nearly ceased in all groups. Stable Whitney groups were still producing 527 (experiment 40) and 691 (experiment 41) aggressive acts per 4 hr on the 16th and 17th days. These data suggest that density is not reduced in such a way that aggression frequencies are equalized. That is, density is insensitive to aggression. This is equally evident in comparisons of emigration rate and aggression frequency for 1970 experiments. The groups showing exceptionally frequent aggression on day 3 (experiments 37, 38) did not subsequently drop faster in size than groups showing much less frequent aggression. Apparently then, the frequency of agonistic behavior as measured by an observer was not tied closely enough to density to effect sensitive density control.

Although I have not presented a breakdown here, I also found that the frequencies of different *types* of aggression were even less closely related to density and rate of emigration than was total frequency of aggressive acts. Some groups showed unusual numbers of bouts involving display or violent physical contact, but these invariably arose from dominance contests between single

pairs of trout. As was stated earlier, such experiences of individuals were not closely related to their subsequent movements.

Discussion

One of the features implicit in my description of stream social structure (Jenkins 1969) was the rarity of strictly territorial individuals. Movement ranges of high- and low-ranking fish overlap considerably, because fish dominant in their movement ranges (i.e., territorial) defend them only sporadically, and do not persist in attacks on familiar subordinates. This description applies to feral populations of rainbow trout as well as groups of wild brown trout. I find a simple, precise form of social density control difficult to visualize in such a social framework. Indeed, within less than 15 m of my study stream I observed a 15-fold range in number of stable occupants resulting from initial differences in density up to 25-fold. The highest density was about 1.3 fish per m^2, but spatial distribution was such that most fish at that density were less than 10 cm from two or more other fish. The rainbow trout in all populations averaged larger than the peak size of wild fish in Convict Creek, and all lost some weight even at the lowest density of 0.09 fish/m^2. Thus, ecologically realistic densities seem to have been exceeded without the occurrence of compensatory dispersal. In fact, the frequency of aggression per individual of both strains showed signs of dropping at the highest densities (see Fig. 6), suggesting that social effects on dispersal might be even less likely at densities higher than those attained here.

Were social density regulation to take place in stream trout populations, it would presumably be related in some way to the frequency of overt aggressive acts among fish with overlapping movement ranges. Although there was an approximate relation between density and aggression, the level of aggression at a given time was not proportional to subsequent reduction in density by emigration. Thus, even if density and aggression had been more closely related, there is no reason to suppose that density regulation would have occurred.

One can readily imagine how domestication or hatchery rearing of trout could debilitate their social density-regulation mechanisms, since regulation must depend on subtle behavioral traits. I have presented evidence elsewhere (Jenkins 1969) that wild brown trout live relatively peacefully at high densities, and that socially subordinate fish are able successfully to procure food and avoid excessive energy expenditure. The same seems to have been true of hatchery fish in the present study. Natural selection must give wild fish extraordinarily acute powers to predict "success" elsewhere if they can be induced to leave such favorable-appearing circumstances for the unknown. Such powers might easily be dulled through relaxation of selection pressures in a hatchery, or by movement restraints at high densities in rearing ponds. Despite these possibilities, we must still consider that social density regulation might be absent in certain stream-resident populations of trout, or perhaps lost at a certain age or size. Emigration might have far more drastic consequences for nonmigratory fish in a fully occupied stream than for anadromous fish, which will eventually leave the stream anyway. If domestication or hatchery-rearing could disrupt density-regulation mechanisms accidentally, natural selection and early experience in some natural habitats conceivably could do the same.

Even though territory defense is not complete, agonistic behavior in settled groups of hatchery and wild fish is largely responsible for spatial distribution within a limited stream area (Jenkins 1969). This raises the question as to why such social distribution does not operate (at least for hatchery trout) over more extensive lengths of stream. Part of the answer seems to be that inter-individual space is an instantaneous feature of a group, while all group members share similar or identical movement ranges over finite periods of time. Spacing exists, but it is not an absolute population quality.

It is understandable that fish living in a fully occupied stream would tolerate subordination within their movement ranges. Complete change of area in such circumstances has definite disadvantages, such as decreased success in aggression in unfamiliar areas (Chapman 1962; Jenkins 1969). In the present study, I saw sporadic incidents where early-dispersing fish drove larger latecomers back to the area of introduction. In this way, social behavior could have stabilized unbalanced density situations rather than preventing them. One-way traps immediately upstream and downstream from the area of introduction might have led to results more compatible with the density-equalization hypothesis, by preventing emigrants from returning after defeat elsewhere.

Chapman's (1962) experiments with juvenile *Oncorhynchus kisutch* at Oak Creek were most nearly comparable to those of the present study, in that he planted different-size groups in identical habitats and ended up with groups of identical size. As in the present study, most of the movement occurred during the first 2 days, but he observed in addition a great deal of compensatory emigration

on following days. Since the Oak Creek habitats were highly artificial, with simplified substrate features, I would expect that the social structures were primarily territorial mosaics (see discussion in Jenkins 1969). If this was the case, more precise density regulation would have been expected than in the complicated habitats and social structures of the present study.

Without compensatory emigration to equalize densities, stream populations of hatchery trout could theoretically be overcrowded to the point of reduced growth rate and compensatory mortality. Brynildson (1967) found that rainbow trout fingerlings stocked in Mt. Vernon Creek dispersed little, and grew slowest in stream areas of highest density. However, in Black Earth Creek, where 10,000 rainbow trout of one strain dispersed about the same as 4000 of another, the fish at high density survived better than low-density fish through the first summer (about 40% vs. 25%) and grew equally well (Brynildson et al. 1966). The strains may have differed in their nonsocial tendencies to move after introduction — as seems to have been the case in the present study — or they may have differed in their social tolerance of density. In either instance, relation of population to food supply appears not to have been critical in Black Earth Creek.

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Fecundity of the Left and Right Ovaries of Grand Bank Capelin (*Mallotus villosus*)

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Fecundity estimates of the left and right ovaries of Grand Bank capelin (*Mallotus villosus*) revealed a considerable disproportion in egg counts, with the left ovary being on the average nine times more fecund than the right. For each ovary the relation between fecundity and length was curvilinear, and between fecundity and age was linear, with the left ovary increasing in fecundity with length and age at a faster rate than the right. Capelin fecundity is related more to age than length, and this was attributed to the inhibition of growth due to spawning and to the presence of repeat spawners in the older fish.

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ALTHOUGH the left and right ovaries of capelin (*Mallotus villosus*) have similar rates of egg development (Winters 1970), the left ovary is several times larger than the right. Such asymmetry in ovary sizes is not uncommon in fishes, yet most fecundity estimates describe only the total fecundity of both ovaries. The total fecundity of Atlantic capelin has been described by Templeman (1948) for Newfoundland coastal waters, by Winters (MS 1966) for the Grand Bank, and by Pozdniakov (1957) for the Barents Sea. Pozdniakov (1957) also lists the range and mean of the fecundity of each ovary separately, but does not show how each is related to fish length or age. This report documents the egg potential of the left and right ovaries of Grand Bank capelin and the relations of each to length and age.

Materials and Methods

Fecundity estimates were determined from prespawning capelin collected by the research vessel *A. T. Cameron* in otter-trawl tows along the southwest slope of Grand Bank in early May 1966. The specimens ranged in total length from 153 to 206 mm, and egg diameters varied from 0.40 to 0.70 mm. The ovaries were separated carefully, removed, and placed in separate vials containing a solution of Gilson's fluid with double the amount of acetic acid (Simpson 1951). After 3–4 weeks preservation in Gilson's fluid, during which the vials were periodically shaken, the ovaries were broken up manually, and by repeated washing and decanting, the tissue debris was removed and the eggs separated from each other. The eggs were then stored in vials containing 5% formalin until they were counted.

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The whirling vessel as modified by Pitt (1965) was used to fraction the egg samples. For the left ovary two random subsamples of $\frac{1}{10}$ each were combined and fractioned again to produce final subsamples of $\frac{1}{10}$. The right ovary was fractioned only once, resulting in subsamples of $\frac{1}{10}$. Two subsamples were enumerated for each ovary, their counts averaged and then multiplied by the appropriate conversion factor to arrive at the total count. No reliability test of the sampling technique was made, since essentially the same method was used by Winters (1966) and found to be quite accurate.

Results

Fecundity estimates of the left and right ovary and both ovaries combined are given in Table 1. The fecundity of the left ovary was 14,800–56,500 eggs with a mean of 30,677 eggs. The fecundity of the right ovary was 1100–6750 eggs with a mean of 3468 eggs. Total fecundity of both ovaries was 16,550–61,500 eggs with a mean of 33,850 eggs. The ratio of the fecundity of the left to the right ovary varied from 3.7 to 21.9 with a mean of 8.8.

Log–log regressions of fecundity on length and fecundity on age were calculated for the left and right ovaries and for both ovaries combined (Fig. 1). Natural logarithms taken to four places of decimals were used in all instances and the fecundity was expressed in thousands of ova. The log of capelin fecundity increased with the log of length according to the following relations:

right ovary — $\log F = 2.54 \log L - 5.0697$ (101 specimens)
left ovary — $\log F = 3.29 \log L - 6.7823$ (103 ")
both ovaries — $\log F = 2.94 \log L - 4.8473$ (114 ").

TABLE 1. Fecundity estimates of the left and right ovaries of capelin, together with combined estimates of both ovaries, Grand Bank, 1966.

Length interval (*mm*)	Left ovary			Right ovary			Both ovaries		
	No.	Range ('000)	Mean ('000)	No.	Range ('000)	Mean ('000)	No.	Range ('000)	Mean ('000)
153–157	4	15.3–27.3	19.1	4	1.3–3.7	2.3	4	17.0–30.9	21.3
158–162	3	20.0–21.0	20.6	3	2.1–3.2	2.8	5	22.1–33.2	25.1
163–167	8	14.8–28.9	21.4	8	1.4–2.7	2.2	10	16.6–31.5	24.5
168–172	6	20.8–30.4	24.0	6	2.6–3.3	3.0	8	24.0–38.6	28.3
173–177	17	20.0–37.2	27.6	16	2.2–5.5	3.6	19	24.2–41.3	31.7
178–182	14	17.3–50.8	31.7	13	2.2–5.2	3.5	16	19.4–55.8	35.4
183–187	20	21.3–42.0	31.8	20	2.1–5.3	3.9	21	23.4–46.4	35.5
188–192	21	19.3–51.0	35.6	21	1.1–5.3	3.5	21	20.4–56.3	39.0
193–197	7	25.8–56.5	40.4	7	2.1–5.5	4.0	7	28.3–61.5	44.4
198–202	2	39.0–42.8	40.9	2	4.3–4.9	4.6	2	33.3–47.6	40.5
203–207	1	45.3	45.3	1	6.8	6.8	1	52.0	52.0
	103	14.8–56.5	30.7	101	1.1–6.8	3.5	114	16.6–61.5	33.8

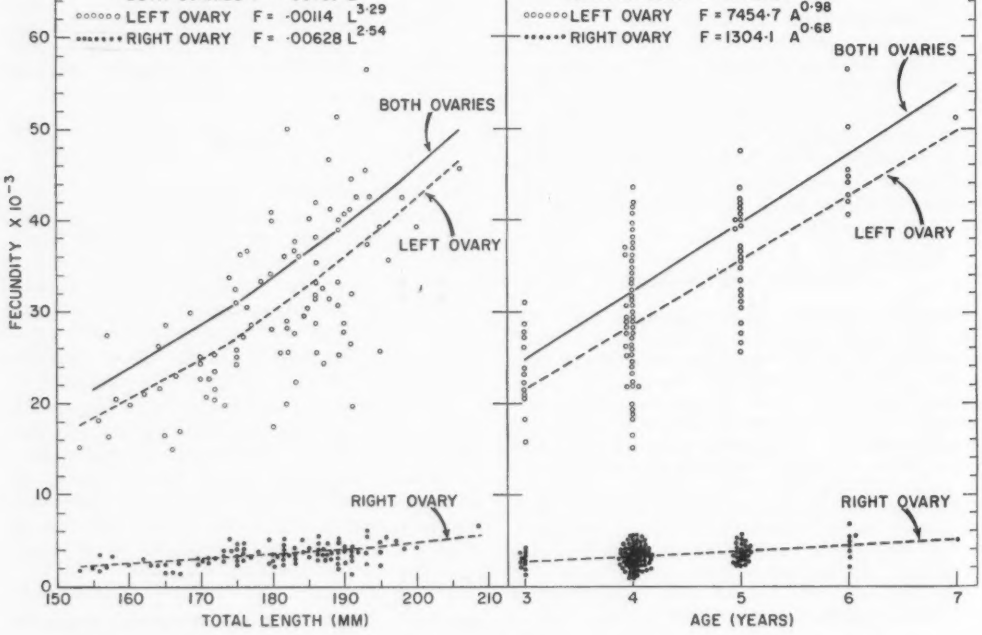
BOTH OVARIES F = ·00785 L^2·94
LEFT OVARY F = ·00114 L^3·29
RIGHT OVARY F = ·00628 L^2·54
BOTH OVARIES F = 8675·9 A^0·95
LEFT OVARY F = 7454·7 A^0·98
RIGHT OVARY F = 1304·1 A^0·68
FECUNDITY X 10^-3
TOTAL LENGTH (MM)
AGE (YEARS)
BOTH OVARIES
LEFT OVARY
RIGHT OVARY

FIG. 1. Fecundity estimates of the left and right ovaries of Grand Bank capelin related to length and age.

The log fecundity–log age relation was as follows:

right ovary — log F = 0.68 log A + 7.1733
left ovary — log F = 0.98 log A + 8.9166
both ovaries — log F = 0.95 log A + 9.0683.

The fecundity of the left ovary increases with fish length and age at a faster rate than the right ovary. Also the disproportion between the left and right becomes increasingly greater with increasing fish length and age.

On the basis of simple correlation coefficients (Table 2) total fecundity and left ovary fecundity are related more to age than length, whereas for the right ovary the fecundity–length correlation coefficient was slightly higher than the fecundity–age coefficient. Since age and length are mutually related, multiple correlation following the method of Croxton (1953) was used to determine the relative contribution of length and age to trends in fecundity (Table 2). For the left ovary and for both ovaries combined, the coefficients of determination are greater for estimating equations employing age only rather than length only, whereas for the right ovary the coefficient of determination using length only is higher. When both length and age are used together, the coefficients of multiple determination are greater than the simple coefficients when age alone was used. This increase in the amount of explained variation in fecundity by the addition of length was not significantly different from 0 in either the left or right ovaries or both ovaries combined (Table 2). Consequently, variation in capelin fecundity can be adequately explained by age alone.

Discussion and Conclusions

Fecundity studies on various species such as the European long rough dab (American plaice), *Hippoglossoides platessoides* (Bagenal 1957), Pacific herring, *Clupea harengus pallasi* (Nagasaki 1958), Atlantic cod, *Gadus morhua* (May 1967), and yellowtail flounder, *Limanda ferruginea* (Pitt 1971) have demonstrated that variations in the fecundity of these species can be adequately explained in terms of length alone. The present study has shown that, for capelin, age rather than length is the dominant factor. This may be explained by the growth, maturity, and spawning characteristics of capelin. Previous studies (Templeman 1948; Pitt 1958) and preliminary assessment of unpublished data indicate that capelin grow rapidly up to the onset of maturity (mainly at ages 3 and 4) after which, growth is considerably retarded and length increments small. Hodder (1963) proposed that fish that have previously spawned have a greater fecundity than fish of the same age and size spawning for the first time, and gave indirect evidence of a possible connection between the frequency of spawning and fecundity of Grand Bank haddock, *Melanogrammus aeglefinus*. In the process of examining capelin otoliths for ageing purposes, the author observed certain irregular zones in the otolith structure that were characterized by an accentuated translucent (winter) band followed by a very narrow opaque (summer) band. A comparison of the marginal zone of the otoliths of recovering-spent capelin at the end of their growing period in November with the marginal zone of the otoliths of pre-spawning, spawning, and recently spent capelin has demonstrated that these irregular zones represent spawning zones, and that the wide winter band and narrow summer band are the result of the pronounced inhibition of growth due to spawning. For the present material, otoliths of fish of each age-group were classified according to the presence or absence of spawning zones, and geometric mean lengths and fecundities were calculated for each group (Table 3). The data indicate that a

TABLE 2. Summary of statistics for multiple correlation of fecundity, age, and length. X_1 = log fecundity, X_2 = log length, and X_3 = log age.

		Left ovary	Right ovary	Both ovaries
No. fish	N	103	101	114
Coeff. of determination for X_1 and X_2	r^2_{12}	0.415	0.257	0.419
Coeff. of correlation for X_1 and X_2	r_{12}	0.644	0.507	0.647
Coeff. of determination for X_1 and X_3	r^2_{13}	0.524	0.230	0.530
Coeff. of correlation for X_1 and X_3	r_{13}	0.724	0.480	0.728
Coeff. of multiple determination	R^2 1.23	0.550	0.281	0.556
Coeff. of multiple correlation	R 1.23	0.741	0.530	0.745
Coeff. of partial determination, X_1 and X_2	r^2 12.3	0.053	0.001	0.058
	t for r 12.3	0.55	0.65	0.54
	P for r 12.3	.50–.60	.50–.60	.50–.60

TABLE 3. Fecundity of Grand Bank capelin at a given age according to the presence or absence of otolith spawning zones.

Age (*years*)	Spawning zone(s) present			Spawning zone(s) absent		
	No.	Geo. mean length (*mm*)	Geo. mean fecundity	No.	Geo. mean length (*mm*)	Geo. mean fecundity
3	4	160	23124	3	172	22392
4	24	172	31222	19	177	27105
5	15	183	40013	11	191	34870
6	7	189	42667	4	194	40065

large proportion of mature female capelin in the present material are repeat spawners, which have a greater fecundity than fish of the same age spawning for the first time. This supports the relation noted by Hodder (1963), and indicates that variation in capelin fecundity within an age-group can be partially explained by the proportion of repeat spawners. The length variation of mature capelin within and between age-groups, however, is very small. Thus, as mature female capelin increase in age and the proportion of repeat spawners also increases without a significant increase in length, variations in fecundity are more affected by age and the presence of repeat spawners than length.

For Barents Sea capelin ranging in fork length from 116 to 176 mm, Pozdniakov (1957) gives a fecundity range of 9478–21,060 eggs (mean = 13,935 eggs) for the left ovary, 69–1122 eggs (mean = 618 eggs) for the right ovary, and 4518–22,021 eggs (mean = 10,764 eggs) for both ovaries combined. These estimates are in each instance considerably lower than those reported here for Grand Bank capelin of similar size, and these differences may be related to differences in environmental conditions in the two areas. However, the mean ratio of the fecundity of the left ovary to the right in Barents Sea capelin (22.5) is much greater than for Grand Bank capelin. Although the range of fecundity is essentially the same, the value of 2.94 found in this paper for the exponent in the length–total fecundity relation is lower than that reported by Winters (MS 1966) (3.27) for Grand Bank capelin of similar size. The higher proportion of older fish in the present data may account for the lower value. For Newfoundland coastal capelin, ranging from 130 to 210 mm total length, Templeman (1947) reports a much lower maximum fecundity (52,000 eggs).

There are several reports in the literature of asymmetrical ovaries in fishes. Brown and Kamp (1942) report that the left ovary of brown trout (*Salmo trutta*) usually contains more eggs than the right and attribute this to the bending of the posterior portion of the intestine to the right, thus crowding the right ovary at its caudal end. A similar size disparity exists between the left and right ovaries of the northern anchovy, *Engraulis mordax* (MacGregor 1968), whereas in sockeye salmon from Kodiak Island, Alaska, the right ovary is more fecund than the left (Rounsefell 1957).

In capelin there is no strong bending of the intestine to the right with consequent crowding of the ovary, and the considerable disproportion between the left and right ovaries cannot be explained on this basis. The left ovary, however, even in immature fish is considerably larger than the right, and this condition accounts for the much greater fecundity of the left ovary.

Acknowledgments

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Quick Statistical Methods for Analysing the Sequences of Fish Counts Provided by Digital Echo Counters

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MacNeill, I. B. 1971. Quick statistical methods for analysing the sequences of fish counts provided by digital echo counters. J. Fish. Res. Bd. Canada 28: 1035–1042.

The data records of digital echo counters consist mainly of sequences of overlapping counts. To estimate target density and the total number of distinct targets present, one should make appropriate allowances for the redundancy induced by overlapping. With this in mind, point and interval estimation methods are suggested for use in the hand analysis of such sequences. The techniques were developed in response to the need for ship-board analysis of data when there is an immediate requirement to gauge variations in target density as fish surveys are being conducted.

MacNeill, I. B. 1971. Quick statistical methods for analysing the sequences of fish counts provided by digital echo counters. J. Fish. Res. Bd. Canada 28: 1035–1042.

Les sondages accoustiques donnent habituellement le nombre de poissons dans une succession de zones non disjointes. Pour calculer la densité et le nombre total des poissons il faut prendre compte de l'empiètement des zones. Nous suggérons des méthodes d'estimation de valeurs et d'intervalles. Nos méthodes numériques sont propres au calcul fait à bord le navire lors même que l'étude sur les variations de densité des poissons se poursuit.

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DIGITAL echo counters for ground fish yield sequences of echo counts from layers located immediately above the sea bed and along a transect. For each pulse of the transducer a count is recorded from each of several layers located immediately above one another. The sonified layers for different pulses overlap to an extent depending upon: (*i*) velocity of the towing vessel; (*ii*) distance of the transducer above the bottom; (*iii*) target strength; (*iv*) physical characteristics of the transducer; and (*v*) the replication (or pulse) rate. This overlapping of the sonified layers contributes to making the totalized counts unrepresentative of the number of distinct targets present, in that the same target may be counted several times. To allow for redundancy in the echo counts we examine the geometry of the sound beam and, under certain assumptions about fish distributions, derive methods that are quick, although not fully efficient, for the point and interval estimation of (*i*) the number of distinct targets present and (*ii*) target density.

The methods that we suggest condense a sequence of counts to a single totalized count. In the case of nonoverlapped data nothing is lost by this, but when overlapping occurs some information concerning the parameters of the target distribution is thrown away by the data reduction, thus reducing the efficiency of the estimators. However, this shortcoming is more than compensated for by ease of computation as is illustrated in the sequel by a numerical example. Hopefully, our analysis of the sound beam may prove to be useful in a wider context.

Some exact small sample and asymptotic theory for the statistical analysis of target counts is given by MacNeill (1971). A digital echo counter has been described by Dowd (1967) and a comparison of the performance of such a counter with that of an echo integrator was given by Dowd et al. (1970). The procedures developed below have been used at the Marine Ecology Laboratory in Dartmouth, N.S. Some aspects of the geometry of the sound beam are discussed by Cushing (1968) and MacNeill (1971). Cushing (1968) has used echo-sounder paper recordings to estimate abundance acoustically.

Geometry of the Sound Beam

As an echo-counting device is towed along a transect, sound pulses are beamed at the bottom at regular intervals. For each pulse in the sequence the echo data from the bottom layer is digitized and recorded as a count in integer mode. The sequence of counts so formed provides us with the

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basis for our analysis. We denote the number of counts (pulses) in the sequence by m; the total sum of the m individual counts by N; the total number of distinct targets actually present in the bottom layers of the sonified volumes by N*; the average depth in meters by R; the number of transmissions per minute by t; the velocity of the towing vessel in meters per minute by v.

It is assumed that the region sonified by a single pulse is the intersection of an elliptic cone and a sphere of radius R and that targets are counted from a layer h meters thick at the bottom of the cone. In the above notation, one finds that the bottom width of this cone of sonification is $2R\sin\theta_2$, where θ_2 is the half power half-angle in the fore and aft plane. θ_1 denotes the half power half-angle in the athwartship plane. The choice of the half-power angles to characterize the beam is somewhat arbitrary since higher gain or interest in smaller targets would suggest that wider angles be used. All that is required for the application of the ensuing analysis is that some cone dimensions be specified. We assume that the successive sonified volumes have the same shape, orientation, and size. The order of overlap for a sequence of counts, which is defined to be the number of consecutive sonified regions that overlap the ith, is denoted by k and is found to be the greatest integer in $(2R\sin\theta_2)/d_1$ where $d_1 = v/t$ is the distance travelled between consecutive transmissions. Let A_i denote the layer sonified by the ith transmission and let $V(A_i)$ denote the volume of A_i. Further, let $V(U_{i=1}^{m} A_i)$ represent the entire volume from which targets are counted for a count sequence of length m. To carry out our analysis we need to discuss the region common to (or in the intersection of) several layers. The results (equations 1, 2, and 3) given below are adaptations of results stated by Parzen (1960 p. 76) in a probability context. The region common to $\{A_{ij}, j=1, 2,\ldots,l\}$ is denoted by $\Omega_{i=1}^{l} A_{ij}$ and its volume by $V(\Omega_{j=1}^{l} A_{ij})$.

Recalling that k denotes the order of overlap, we let

$$B_1 = \sum_{i=1}^{m} V(A_i),$$

$$B_2 = \sum_{i_1} \sum_{i_2} V(A_{i_1} \cap A_{i_2}),\ 1 \le i_1 \le i_2 \le m,\ i_2 - i_1 \le k$$

$$\cdot$$

$$\cdot$$

$$B_l = \sum_{i_1} \cdots \sum_{i_l} V(\Omega_{j=1}^{l} A_{ij}),\ 1 \le i_1 < i_2 < \ldots < i_l \le m,\ i_l - i_1 \le k$$

$$\cdot$$

$$\cdot$$

$$B_{k+1} = \sum_{i=1}^{m-k} V(\Omega_{j=0}^{k} A_{i+j}). \quad (1)$$

In this notation it can be shown that:

$$V(U_{i=1}^{m} A_i) = \sum_{j=1}^{k+1} (-1)^{j+1} B_j. \quad (2)$$

A certain part of the total region, $U_{i=1}^{m} A_i$, will be sonified exactly once, another part will be sonified exactly twice, and so on. If we denote by M_i, $i=1, 2,\ldots,k+1$, the volume of the part of $U_{i=1}^{m} A_i$ that is sonified exactly i times, then it can be shown that

$$M_i = \sum_{j=i}^{k+1} (-1)^{j-i} \binom{j}{i} B_j \quad i=1,2,\ldots,k+1 \quad (3)$$

and that

$$\sum_{i=1}^{k+1} M_i = V(U_{j=1}^{m} A_i).$$

In other words, we have subdivided the total region that is subjected to the echo-counting device towed along a transect into $k+1$ disjoint subregions such that a target in the ith of these subregions will be sonified i times, will be subject to being counted i times, and hence will be included in the totalized count i times. It is this redundancy that makes N, the totalized count, unrepresentative of N*, the total number of distinct targets actually present in the sonified region.

The proportion of $V(U_{j=1}^{m} A_j)$ subject to being sonified i times is denoted by P_i and is defined to be

$$P_i = M_i / V(U_{j=1}^{m} A_j)\ i=1,2,\ldots,k+1. \quad (4)$$

The parameters defined by equation 4 are used in the next section for developing estimation methods. To evaluate equation 4 we must be able to compute the volume of the layers, $V(A_i)$, and overlap volumes such as $V(A_i \cap A_{i+j} \cap A_{i+j+l})$ or $V(A_i \cap A_{i+j+l})$. In Appendix 1, I give formulae for calculating $V(A_i)$ and $V(A_i \cap A_{i+j})$, $j \le k$. $V(A_i)$ is a function of R, θ_1, θ_2, and layer thickness. $V(A_i \cap A_{i+j})$ has an additional parameter, namely, distance travelled between the ith and the $i+j$th pulse. Now, it is always true that $A_i \cap A_{i+j+l} \supseteq A_i \cap A_{i+j} \cap A_{i+j+l}$, but, in addition, $A_i \cap A_{i+j+l} = A_i \cap A_{i+j} \cap A_{i+j+l}$ under special circumstances (e.g., the A_i's are convex). In the event that the beam angles are small and the layer thickness is sufficiently large compared with depth, we find

that, although $A_i \cap A_{i+j+l}$ does not precisely coincide with $A_i \cap A_{i+j} \cap A_{i+j+l}$, the coincidence is close enough that one can consider the volumes as good approximations of one another. These hypotheses are satisfied by the echo-counting device reported by Dowd (1967). We restrict our attention to such devices and hence do not feel the need to compute the precise volume of the intersection of more than two sonified layers. These simplifying assumptions enable us to put equations 2, 3, and 4 into forms more easily evaluated. We let p_j represent the proportion of a layer overlapped by one j pulses later.

Thus:

$$p_j = V(A_i \cap A_{i+j})/V(A_i),$$

$$q_j = 1-p_j \quad j=1,2,\ldots,k.$$

In this notation and under the simplifying assumptions that imply that

$$V(\cap_{j=1}^{l} A_{ij}) \simeq V(A_{\min_j i_j} \cap A_{\max_j i_j})$$

one can show that

$$V(U_{i=1}^{m} A_i) \simeq (m\, q_1 + p_1)\, V(A_i)$$

$$\simeq m\, q_1\, V(A_i) \qquad (5)$$

and that

$$M_1/V(A_i) \simeq 2q_1 + (m-2)\,(2q_1 - q_2) \simeq m(2q_1 - q_2)$$

$$M_j/V(A_i) \simeq 2(q_j - q_{j-1}) + (m-1-j)\left[(q_j - q_{j-1}) - (q_{j+1} - q_j)\right]$$

$$\simeq m\left[(q_j - q_{j-1}) - (q_{j+1} - q_j)\right] \qquad 1 < j \leq k-1, \qquad (6)$$

$$M_k/V(A_i) \simeq 2(q_k - q_{k-1}) + (m-1-k)\left[(q_k - q_{k-1}) - p_k\right]$$

$$\simeq m\left[(q_k - q_{k-1}) - p_k\right],$$

$$M_{k+1}/V(A_i) \simeq (m-k)p_k \simeq m\, p_k,$$

which means that, for large m,

$$P_1 \simeq (2q_1 - q_2)/q_1,$$

$$P_j \simeq \left[(q_j - q_{j-1}) - (q_{j+1} - q_j)\right] / q_1 \quad 1 < j \leq k-1, \qquad (7)$$

$$P_k \simeq \left[(q_k - q_{k-1}) - p_k\right] / q_1,$$

$$P_{k+1} \simeq p_k / q_1.$$

Estimation Methods

If it is assumed that targets are distributed throughout a region according to a homogeneous Poisson process, then, given that there are N^* distinct targets present in the subregion to be sonified, one may assume that they are conditionally distributed according to a uniform distribution, i.e., the expected number of targets present in a part of the sonified subregion is to N^* as the volume of the part is to that of the whole subregion. Consequently, the totalized echo count, N, will vary depending upon the particular configuration of the N^* distinct targets and, for N^* fixed, the obvious estimator for N is $\hat{N}$ where

$$\hat{N} = E[N|N^*] = \sum_{i=1}^{k+1} i[N^* P_i] \simeq \frac{N^*}{q_1}. \qquad (8)$$

The last part of equation 8 follows from equation 7. An assumption made here is that if a target is present in a sonified volume it will be detected and counted. More is said about this assumption in the last section.

It is interesting to note that $\hat{N}$ is essentially independent of the proportions of overlap except for that of the first. This makes for a very simple relation between the actual number of targets and the expected or mean count regardless of the order of overlap. Such a simple relation does not hold for the higher moments. Equation 8 gives the expected value of N given N^*,N being a random variable and N^* being a parameter. This suggests $\hat{N}^*$ as a point estimator for N^* given N where

$$\hat{N}^* = q_1 N. \qquad (9)$$

For large m, and under the assumption made in the previous section concerning beam angles and layer thickness, $\hat{N}^*$ will have small bias for estimating N^*.

We now compute the variance of N given N^*, which we denote by Var $(N|N^*)$. It can be noted that

$$N = \sum_{i=1}^{k+1} i\, N_i \qquad (10)$$

where N_i is the random variable indicating the number of targets found in M_i, that is, in the region subject to i sonifications. We also have that

$$N^* = \sum_{i=1}^{k+1} N_i. \tag{11}$$

Under the assumptions made concerning the underlying distribution of the targets, N_i, $i = 1, 2, \ldots, k+1$, subject to equation 11, are multinomially distributed with parameters given by equations 4 or 7. Equation 10 then implies that

$$\begin{aligned}\mathrm{Var}[N|N^*] &= \sum_{i=1}^{k+1} i^2 \,\mathrm{Var}(N_i) + 2\sum_{i=2}^{k+1}\sum_{j=1}^{i-1} ij \,\mathrm{Cov}\,(N_i, N_j) \\ &= \sum_{j=1}^{k} (k+1-j)^2 \,\mathrm{Var}(N_j) + 2\sum_{j=1}^{k-1}\sum_{l=j+1}^{k} (k+1-j)(k+1-l)\,\mathrm{Cov}(N_j, N_l) \end{aligned} \tag{12}$$

where

$$\mathrm{Var}(N_j) = N^* P_j (1 - P_j)$$

and

$$\mathrm{Cov}(N_j, N_l) = -N^* P_j P_l.$$

One should note that, although $\mathrm{Var}(N|N^*)$ is a function of N^*, $N^{*-1}\,\mathrm{Var}(N|N^*)$ is independent of N^*.

As indicated above, precise distribution theory is available; but for practical reasons, and since, for large samples of the type encountered in echo counting, the central limit theorem applies, we will base interval estimation for N^* upon the following approximate probabilistic statement:

$$P\left[\left|N - \frac{N^*}{q_1}\right| \le 2\sqrt{\mathrm{Var}(N|N^*)}\right] \approx 0.95,$$

which implies that

$$P\left[q_1 N - 2q_1\sqrt{\mathrm{Var}(N|N^*)} \le N^* \le q_1 N + 2q_1\sqrt{\mathrm{Var}(N|N^*)}\right] \approx 0.95.$$

Having replaced N^* by $\hat{N}^* = q_1 N$, one obtains the following as approximate 95% confidence limits for N^*:

$$q_1 N \pm 2q_1 \sqrt{q_1 N\left(\frac{1}{N^*}\,\mathrm{Var}(N|N^*)\right)}. \tag{13}$$

The estimate of the total number of distinct targets present is not the only statistic that is of interest. An estimate of $\hat{N}^*$ targets present for a depth of 20 m would be much more impressive than the same estimate for a depth of 100 m. This follows from the fact that the volume of sonification is quite small for the 20-m depth and quite large for the 100-m depth. Consequently, a measure of target density would be of value in comparing the counts from different regions. If we denote target density by F and let V(A) denote layer volume, then we may estimate F by $\hat{F}$ where

$$\hat{F} = \frac{N}{m}\,(V(A))^{-1}. \tag{14}$$

$\hat{F}$, the estimator that intuition suggests, can be used as an estimator of the intensity parameter of the underlying Poisson distribution that has been assumed.

The justification for equation 14 is as follows:

$$F = \text{target density} = \frac{\text{number of distinct targets present}}{\text{total volume of sonification}}.$$

Using estimators derived earlier, we obtain

$$\hat{F} \approx \frac{N q_1}{V(A)\,(m\,q_1 + p_1)} \approx \frac{N q_1}{m\,V(A) q_1} = \frac{N}{m\,V(A)}.$$

Since

$$E\left[\hat{F}|N^*\right] \simeq \frac{1}{m\,V(A)}\,E\left[N|N^*\right] \simeq \frac{N^*}{q_1 m\,V(A)} \tag{15}$$

and

$$\mathrm{Var}\left[\hat{F}|N^*\right] \simeq \mathrm{Var}\left[\frac{N}{m\,V(A)}\,\middle|\,N^*\right] = \frac{\mathrm{Var}[N|N^*]}{m^2\,(V(A))^2} \tag{16}$$

we may obtain an approximate 95% confidence interval for F from the following probabilistic statement:

$$P\left[|\hat{F} - F| \le 2\sqrt{\mathrm{Var}(\hat{F}|N^*)}\right] \approx 0.95,$$

which implies that

$$P\left[\frac{N}{m\,V(A)} - \frac{2\sqrt{\mathrm{Var}(N|N^*)}}{m\,V(A)} \le F \le \frac{N}{m\,V(A)} + \frac{2\sqrt{\mathrm{Var}(N|N^*)}}{m\,V(A)}\right] \approx 0.95.$$

As in obtaining confidence intervals for N*, one can replace Var [N | N*] with $q_1 N(\frac{1}{N^*} \quad \mathrm{Var}(N|N^*))$ to obtain the following approximate 95% confidence interval for F:

$$\frac{N}{m\,V(A)} \pm \frac{2}{m\,V(A)} \sqrt{q_1 N\left(\frac{1}{N^*}\,\mathrm{Var}(N|N^*)\right)}. \quad (17)$$

Sometimes separate sequences of counts are obtained simultaneously from several sonified layers one above the other. We subscript the statistics and parameters for the bottom layer with b (e.g., N_b), for the upper layer with u (e.g., q_{1u}) and for the combined layer by c (e.g., $\hat{N}_c^*$). Then, provided we can assume the layers to be statistically independent, one can use the computations for the separate layers to obtain point and interval estimates for the combined volume. For example, the appropriate interval estimate for N_c^* is found to be

$$N_b q_{1b} + N_u q_{1u} \pm 2\sqrt{\left(q_{1b}^3 N_b \frac{\mathrm{Var}(N_b|N_b^*)}{N_b^*} + q_{1u}^3 N_u \frac{\mathrm{Var}(N_u|N_u^*)}{N_u^*}\right)}.$$

Discussion

We have claimed in the title that the estimation methods are quick. This will be so only if one prepares beforehand a set of tables specific to the transducer being used. Different beam angles, replication rates, and layer thicknesses will require different tables. To estimate target density using equation 14, all that one requires is V(A). This can be obtained precisely from equation 18 in Appendix 1, but numerical methods are required for its evaluation. Consequently, for a fixed set of parameters (h,θ_1,θ_2) one requires a table of volumes for a range of depths (R), a simple job for a computer. It is pointed out in Appendix 1 that Cushing (1968) gives a close and simple approximation to equation 18 that is especially good for small differences in the beam angles. To obtain point estimates of the number of distinct targets, one requires q_1 in addition to V(A). q_1 is obtained using equations 21 or 22 again with the help of numerical integration. q_1, for fixed (h,θ_1,θ_2), depends not only on the depth (R) but also on the distance travelled between pulses. Consequently, a two-dimensional array is required to adequately tabulate q_1. The interval estimates require knowledge of $N^{*-1}\,\mathrm{Var}(N|N^*)$ as well as Vol and q_1. The tabulation again requires a two-dimensional array.

To summarize, we have proposed point estimators given by equations 9 and 14 and interval estimators given by equations 13 and 17, but to easily use these four expressions one must prepare beforehand tables of V(A), q_1, and $N^{*-1}\,\mathrm{Var}(N|N^*)$ using the formulae given in Appendix 1.

To illustrate the techniques developed above, we present a numerical example. The data used is a small portion of a sequence of counts obtained in the Gulf of St. Lawrence during the summer of 1968. The data were collected with a digital echo-counting appartus designed and built by the Marine Ecology Laboratory in Dartmouth, N.S. For a description of the counter, refer to Dowd (1967). A 5-min portion of a run at 6 knots yielded a totalized count of 340 echos for the bottom 4-m layer. The average depth below the transducer was 49.5 m, the beam parameters were $\theta_1 = 7°$ and $\theta_2 = 3.5°$, and the replication rate was 96 pulses/min. The tables used were based on spacings of 1 m for depth and 15 m/min for velocity. Then $m = 5 \times 96 = 480$ and $v \approx 185$ m/min. Using linear interpolation we estimated q_1 by the following scheme:

$$\left.\begin{array}{l} q_1(49,180) = 0.4087 \\ q_1(50,180) = 0.4006 \end{array}\right\} \tfrac{1}{3} \Rightarrow 0.4047$$

$$\left.\begin{array}{l} q_1(49,195) = 0.4412 \\ q_1(50,195) = 0.4325 \end{array}\right\} \tfrac{1}{3} \Rightarrow 0.4369$$

$$\left.\begin{array}{l} 0.4047 \\ 0.4369 \end{array}\right\} \tfrac{1}{3} \Rightarrow 0.4154 \cong q_1.$$

It should be noted that equation 21 rather than 22 was used in the computation of overlap volumes. Interpolating as above, it was found that $V(A) \approx 211.6\ m^3$ and $N^{*-1}\,\mathrm{Var}(N|N^*) \approx 0.5142$. Thus, using equation 9, one obtains as a point estimate for N*

$$\hat{N}^* = 141.24 \approx 141.$$

This is substantially different from the totalized count. The 95% confidence interval for N* obtained from equation 13 is found to be 141.24 ± 7.08. The point estimate for F using equation 14 is $\hat{F} = 0.003348$, and the interval estimate obtained from 17 is 0.003348 ± 0.000168. These numbers can be rounded off to avoid the impression of more precision than is actually present.

Caveat

A word or two of caution in interpreting the results is in order at this time. A number of approximations have entered into the computations and are itemized below.

(*i*) Approximations for M_j, $j=1,2,\ldots k+1$ are suggested in equation 7. The approximation will have little effect provided m is large ($m > 100$), the beam angles are small, and the layer thickness is not too small in relation to the depth.

(*ii*) An underlying homogeneous Poisson process was assumed to describe the target distribution. For m not too large and for low target density, this was shown by MacNeill (1971) to be a reasonable assumption. However, it is always possible to choose m large enough to have this assumption violated.

(*iii*) Digital echo counters effect a censoring of the data. The consequences of this have been ignored in the preceding analysis. It is felt that censoring will not have much effect on low concentrations but will be a significant factor for high density.

(*iv*) It has been assumed that a detectable target is counted as long as it is in the bottom layer of the cone of sonification defined by the half power half-angles and is not counted if outside. The edges of the cone are actually quite fuzzy and target strength diminishes continuously as the directivity angle increases (until the first minimum is reached, side lobes being regarded as unimportant). This suggests that a target that is counted when located at the center of the cone might not be counted near the edge and that large targets outside the cone may be counted. This point is regarded as important but it will be necessary to take into account the size distribution of target strengths to make the proper allowances. Another factor disturbing the assumption that detectable targets are counted if, and only if, they are present in the bottom layers of the sound cone is the interaction of the echos of two or more targets, each located roughly the same distance from the transducer. Several echos of less than threshold strength can combine to yield a count and echos of greater than threshold strength can interfere to yield no count.

(*v*) It has been assumed that estimators are roughly normally distributed in calculating confidence intervals such as is given by equation 17. This assumption is reasonable enough if the sequence length is large enough and yet not so large that different parts of the range have significantly different target densities.

(*vi*) In calculating confidence intervals one must use $\mathrm{Var}(N|\hat{N}^*)$ instead of $\mathrm{Var}(N|N^*)$ since N^* is unknown (otherwise there is no point in estimating it). For long data sequences this is not regarded as an important problem ($m > 100$).

(*vii*) In computing confidence intervals for combined layers it has been assumed that the layers are statistically independent. The validity of this assumption is unchecked.

In summary, the methods outlined should be useful in interpreting target density provided the number of counts analysed at one time is not too small (100 or more) nor so large that the parameters of the underlying target distribution change from one part of a run to another.

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Appendix 1

We give below the details for computing the volume of a sonified layer, A_i, and of the intersection of two sonified layers, $A_i \cap A_{i+j}$. The formulae, which are essential for using the estimation methods proposed above, are not in simple closed form. However, they have been put into forms that lend themselves to easy evaluation by computer using standard numerical methods. They should be considered as algorithms to be used in the preparation of tables, which can then be used for the quick and immediate hand analysis of sequences of data counts. Also, these complicated but precise formulae can be used to appraise simple closed-form approximations.

Since these volumes are initially expressed as integrals with limits that are sometimes rather complicated, we begin our discussion by establishing a more concise notation for certain formulae that appear in these limits. We define:

$$\rho(\theta,\varphi|d) = d/(2\sin\theta\sin\varphi);$$

$$f(\theta|R,d) = \arcsin[d/(2R\sin\theta)];$$

$$g(\theta|\theta_1,\theta_2) = \operatorname{arc\,cot}\left(\frac{\cos^2\theta}{\tan^2\theta_1} + \frac{\sin^2\theta}{\tan^2\theta_2}\right)^{\frac{1}{2}};$$

$$h(\theta,\varphi|d,h,R) = d\sin\theta\sin\varphi + ((R-h)^2 - d^2(1-\sin^2\theta\sin^2\varphi))^{\frac{1}{2}};$$

$$\theta_0(R,d,\theta_1,\theta_2) = \operatorname{arc\,cot}\left\{\frac{\sin\theta_1}{\sin\theta_2}\left[\frac{4R^2}{d^2}\sin^2\theta_2 - 1\right]^{\frac{1}{2}}\right\};$$

$$d' = \frac{d^2 - h^2 + 2Rh}{d};$$

$$\chi^2 = \left(R^2 - \left(\frac{d'}{2}\csc\theta_2\right)^2\right)\sin^2\theta_1,$$

$$\theta_1(R,d,\theta_1,\theta_2) = \operatorname{arc\,cot}\frac{2\chi}{d'}.$$

In this notation it can be shown that the volume of water in the entire cone of sonification (a function of R, θ_1, and θ_2) is given by

$$V(R,\theta_1\theta_2) = 4\int_0^{\pi/2}\int_0^{g(\theta|\theta_1,\theta_2)}\int_0^{R}\rho^2\sin\varphi\,d\rho\,d\varphi\,d\theta$$

$$= \frac{2\pi}{3}R^3 - \frac{4R^3}{3}\int_0^{\pi/2}\cos[g(\theta|\theta_1,\theta_2)]d\theta. \quad (18)$$

The volume of a layer h units thick, which we have previously denoted by $V(A_i)$, is then given by

$$V(A_i) = V(R,\theta_1,\theta_2) - V(R-h,\theta_1,\theta_2). \quad (19)$$

This volume may be evaluated to the desired level of accuracy by numerical integration of the single integral in equation 18, a simple job for a computer. If the cone is circular, then $\theta_1 = \theta_2$ and equation 18 reduces to $2/3\pi R^3(1-\cos\theta_1)$. Cushing (1968) gives, as an approximation to equation 18, the quantity $2/3\pi R^3(1-\cos\sqrt{\theta_1\theta_2})$. This approximation is particularly good for small differences in the beam angles.

The computation of the volume of overlap of two intersecting layers, which we have denoted by $V(A_i \cap A_{i+j})$, is now discussed. If d is the distance travelled between two pulses, then the volume of overlap of the two sonified cones is given by:

$$O(R,\theta_1,\theta_2,d) = 4\int_{\theta_0(R,d,\theta_1,\theta_2)}^{\pi/2}\int_{f(\theta|R,d)}^{g(\theta|\theta_1,\theta_2)}\int_{\rho(\theta,\varphi|d)}^{R}\rho^2\sin\varphi\,d\rho\,d\varphi\,d\theta$$

$$= 4\int_{\theta_0(R,d,\theta_1,\theta_2)}^{\pi/2}\left\{R^3/3\left[\cos(f(\theta|R,d)) - \cos(g(\theta|\theta_1,\theta_2))\right]\right.$$

$$\left.\frac{-d^3}{24\sin^3\theta}\left[\cot(f(\theta|R,d)) - \cot(g(\theta|\theta_1,\theta_2))\right]\right\}d\theta$$

$$\text{if } 0 \le d/2 \le R\sin\theta_2$$

$$= 0$$

$$\text{otherwise.} \quad (20)$$

Again, the single integral in equation 20 can be evaluated easily using numerical techniques. However, we are interested in the volume of the intersection of the bottom layers rather than the entire cones and, for most instances, this can only be obtained approximately from equation 20. We will give the formulae for the precise computation but, for small beam angles and h not too small in relation to R, an adequate approximation to the intersection volume is given by:

$$V(A_i \cap A_{i+j}) \approx O(R,\theta_1,\theta_2,d) - O(R-h,\theta_1,\theta_2,d)$$

$$\text{if } 0 < d/2 \le (R-h)\sin\theta_2$$

$$= O(R,\theta_1,\theta_2,d)$$

$$\text{if } (R-h)\sin\theta_2 < d/2 \le R\sin\theta_2$$

$$= 0$$

$$\text{otherwise.} \quad (21)$$

We have used equation 21 in the numerical example.

The precise volume of overlap is given in terms of I_1, I_2, and I_3 where

$$I_1 = 4\int_{\theta_0(R-h,d,\theta_1,\theta_2)}^{\theta_0(R,d,\theta_1,\theta_2)}\int_{f(\theta|R,d)}^{g(\theta|\theta_1,\theta_2)}\int_{\rho(\theta,\varphi|d)}^{R}\rho^2\sin\varphi\, d\rho\, d\varphi\, d\theta$$

$$+ 4\int_{\theta_0(R-h,d,\theta_1,\theta_2)}^{\pi/2}\int_{f(\theta|R,d)}^{f(\theta|R-h,d)}\int_{\rho(\theta,\varphi|d)}^{R}\rho^2\sin\varphi\, d\rho\, d\varphi\, d\theta,$$

$$I_2 = 4\int_{\theta_0(R-h,d,\theta_1,\theta_2)}^{\min\{\pi/2,\theta_1(R,d,\theta_1,\theta_2)\}}\int_{f(\theta|R-h,d)}^{g(\theta|\theta,\theta_2)}\int_{h(\theta,\varphi|d,h,R)}^{R}\rho^2\sin\varphi\, d\rho\, d\varphi\, d\theta,$$

and

$$I_3 = 4\int_{\min\{\pi/2,\theta_1(R,d,\theta_1,\theta_2)\}}^{\pi/2}\int_{f(\theta|R-h,d)}^{f(\theta|R,d')}\int_{h(\theta,\varphi|d,h,R)}^{R}\rho^2\sin\varphi\, d\rho\, d\varphi\, d\theta.$$

Then

$$V(A_i \cap A_{i+j}) = I_1 + I_2 + I_3$$

$$\text{if } 0 < d \leq R\sin\theta_2 + ((R-h)^2 - R^2\cos^2\theta_2)^{\frac{1}{2}}$$

$$= I_1 + I_2$$

$$\text{if } R\sin\theta_2 + ((R-h)^2 - R^2\cos^2\theta_2)^{\frac{1}{2}} < d \leq 2(R-h)\sin\theta_2$$

$$= O(R,\theta_1,\theta_2,d)$$

$$\text{if } 2(R-h)\sin\theta_2 < d \leq 2R\sin\theta_2$$

$$= 0$$

otherwise. (22)

The integrals, I_1, I_2, and I_3, with some effort, can be reduced to forms involving single integrals as was done with equations 18 and 20, and then numerical techniques can be applied effectively.

Determination of Nitrilotriacetic Acid in Inland Waters by Gas Chromatography of the Trimethyl Ester

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MURRAY, D., AND D. POVOLEDO. 1971. Determination of nitrilotriacetic acid in inland waters by gas chromatography of the trimethyl ester. J. Fish. Res. Bd. Canada 28: 1043–1047.

A method for the analysis of low concentrations of nitrilotriacetic acid (NTA) in natural waters was developed. The NTA was converted to the trimethyl ester and determined quantitatively by gas chromatography using methyl heptadecanoate as an internal standard. The lower limit of detection was 25 μg/liter of NTA. The identity of the ester was confirmed using a mass spectrometer–gas chromatograph combination.

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THE development of a sensitive and specific method for the analysis of nitrilotriacetic acid (NTA) in natural waters was necessitated by its suggested use as a replacement for polyphosphates in detergents.

Many complexometric methods have been developed to measure NTA using different methods of detection. Bouveng et al. (1968) used a specific ion electrode in a potentiometric method and Daniel and LeBlanc (1959) used a polarographic technique. The zinc–zincon method of Thompson and Duthie (1968) used a colorimetric technique, and Fuhrman et al. (1966) and Swisher et al. (1967) used complexometric titration methods. These methods are substantially based on the chelating properties of NTA and, when applied to natural waters, which contain many cations capable of forming coordination compounds as well as metal-binding substances, these methods often present problems regarding sensitivity and specificity. The methods of Doran (1961, paper electrophoresis), Sykora and Eybl (1968, paper chromatography), and Heinerth (1968, thin-layer chromatography) can give trouble with quantitative analysis and interference from natural substances.

The present technique, however, depends on the well-known chemical property of esterification to give a volatile derivative that can be measured by gas chromatography. The method does not depend on chelation properties, is free of interference from substances present in natural waters, and permits the measurement of NTA in concentrations from 25 μg/liter upwards with a coefficient of variation of ±4%.

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Materials

The nitrilotriacetic acid and dimethyl sulphite were obtained from Eastman Organic Chemicals. Elemental analysis of the NTA was in agreement with the theoretical figures to give 99.5% purity. The methanol–HCl reagent was prepared from Fisher reagent grade methanol and dry HCl gas from lecture bottles supplied by Matheson and Co. The dry HCl gas was bubbled into the methanol to give the calculated increase in weight for a 2 N solution. Since refrigeration would cause condensation during use and possible contamination from water, this reagent was stored at room temperature. As this may also result in the slow formation of methyl chloride and water, fresh reagent was prepared weekly. The internal standard was prepared by carefully weighing out 4.65 mg of methyl heptadecanoate, supplied by Applied Science Labs., and dissolving it in 25 ml of reagent grade chloroform BDH Analar. It was stored in a glass-stoppered vial under refrigeration. Stabilized ethylene glycol adipate was supplied by Analabs and 120–140 mesh Chromosorb W by Johns Manville. A Büchi Rotatory evaporator, Virtis freeze-dryer manifold, Welch Vacuum pump, and a Haake thermostatically controlled water bath were used in forming derivatives from the samples. The vacuum pump was protected from HCl corrosion by a large soda lime trap. Glass ampoules or vials were made as shown in Fig. 1.

GAS CHROMATOGRAPHY

A dual column Perkin-Elmer 900 gas chromatograph was used in a single column mode, in conjunction with a Hewlett Packard recorder (Model No. 7127A) fitted with a Disc integrator. A selector switch was installed to connect the output from either electrometer with the recorder. This permitted later peaks to be eluted without interfering with successive analyses. The columns used were 12 ft long × 1.7 mm I.D. glass-coiled tubing and were packed with 2% ethylene glycol adipate

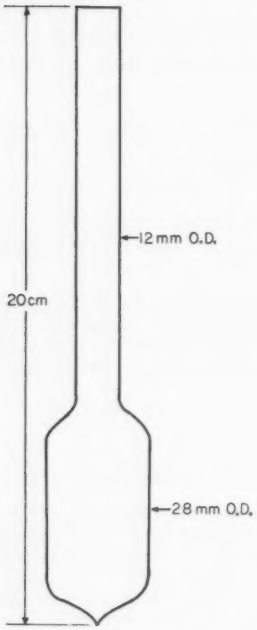

FIG. 1. Pyrex glass ampoule for the preparation of nitrilotriacetic acid derivative.

coated on Chromosorb W. The columns were conditioned overnight at 240 C with nitrogen flowing at 25 ml/min.

Operating conditions

Injector block	210 C
Manifold and detectors	210 C
Columns (isothermal)	195 C
Carrier gas (nitrogen)	25 ml/min
Hydrogen	35 ml/min
Air	400 ml/min

MASS SPECTROMETER–GAS CHROMATOGRAPHY

Conditions were as above with the following exceptions: a $6' \times \frac{1}{8}''$ stainless steel column packed with 2% F.F.A.P. on Chromosorb W was used in the gas chromatograph for convenience and to minimize bleeding. Helium was used as the carrier gas.

Mass spectrometer operating conditions

Ionizing voltage	30 ev
Ionizing current	100 μamp
Ion source temp	250 C
Accelerating voltage	3 kv

Method

Samples of lake water containing 20–2000 μg/liter of NTA were filtered through glass fiber filter paper (Whatman GF/C, 2–3 μ pore size) using a Millipore filter assembly. The filter was changed between samples and during filtration if it became clogged. The samples were then acidified with 2 ml concentrated HCl per 250-ml sample and kept frozen in screw-capped plastic bottles until analyzed. Samples have been kept frozen for 6 weeks and no change in NTA concentration was found. Immediately prior to analysis the samples were thawed in warm tap water and shaken. Portions, 10 ml in volume, were pipetted into the vials and a further 0.1 ml of concentrated HCl was added, since it was found by experience that acidified samples were essential for complete esterification. Possibly an NTA·HCl salt was formed during this step. The sulphate salts of the polycarboxylic amino acids have been found to be better starting compounds for esterification (U.S. Patents 2.428.353 (1947), and 2.532.392 (1950)). The ampoules were then shell frozen in a dry-ice acetone mixture and lyophilized on the freeze-dryer. When the vials were dry, 1 ml of the methanol–HCl reagent and 0.2 ml of dimethyl sulphite were added (Cruikshank and Sheehan 1964). The vials were then sealed and placed in a water bath at 68 C for 3 hr. After esterification, the vials were opened and the excess reagents removed on the rotary evaporator. Finally 20 μliters of the internal standard was added, followed by about 150 μliters of chloroform to wash down the internal standard and dissolve the NTA ester. The vials were then shaken with the aid of a vortex mixer.

The solution was immediately injected into the gas chromatograph, the peak areas measured, and the ratio between the NTA and the internal standard calculated. The concentration was obtained from a calibration graph.

Calibration

A calibration curve was made by taking aliquots of a standard solution of NTA prepared by dissolving 2.0 mg in 0.5 ml 0.1 N NaOH, adding a few ml of water, and acidifying with 2 ml of concentrated HCl before making up to 100 ml. The aliquots were freeze-dried, esterified, and analyzed as described, using the same volume of internal standard throughout. The ratios between the NTA and the internal standard were plotted against concentration of NTA. A linear relation was found over the 0–2000 μg/liter range. For higher values it was found expedient to make a dilution to bring the concentration within the range of the method. The equation for the regression of y on x was given by $y = -10.52 + 1.04x$ where y = the ratio of the NTA peak area to the internal standard peak area, and x = μg NTA/liter. The correlation coefficient was 0.998 and the relative standard deviation was ±4.12% at 560 μg NTA/liter concentration.

Discussion

Previous experience with analysis of the N-trifluoroacetyl methyl esters of amino acids by gas chromatography was used to develop a similar technique for NTA. Esterification of NTA was found to be at a maximum after 3 hr at 68 ± 0.3 C using the methanol hydrochloric acid and dimethyl sulphite mixture. Shorter esterification times gave rise to later peaks on the chromatogram that were thought to be the mono- and di-methyl esters of NTA. After 3 hr esterification these peaks had disappeared to give only one trimethyl ester peak at maximum yield. Further studies are being undertaken to determine if shorter times and higher temperatures can achieve the same degree of esterification without decomposition. Present conditions permit one person to analyze about 10 samples per day.

The possibility that other compounds may coincide with the NTA peak was investigated. The esters of saturated, unsaturated, and branched chain fatty acids were chromatographed with the NTA trimethyl ester derivative (Fig. 2). Interference was found with the C_{18} *iso* compound. However, the presence of fatty acids from natural sources would be shown by a peak for methyl stearate C_{18} or palmitate C_{16} since these are more common by-products of bacterial action. In this instance the use of another gas chromatographic column to alter the relative retention times would overcome this difficulty.

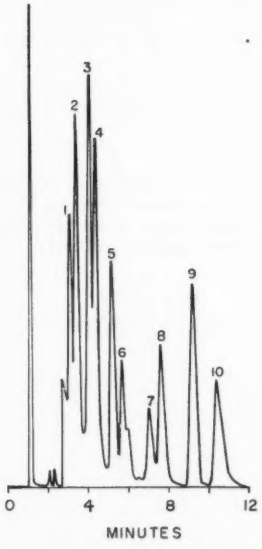

FIG. 2. Chromatogram of fatty acid methyl esters under conditions described in the text. 1, C_{14} *iso*; 2, C_{14}; 3, C_{15} *anteiso*; 4, C_{15}; 5, C_{16} *iso*; 6, C_{16}; 7, C_{17} *anteiso*; 8, C_{17}; 9, NTA + C_{18} *iso*; 10, C_{18}.

Amino acids, carbohydrates, and EDTA do not interfere under the present conditions since they do not form sufficiently volatile derivatives. Possible breakdown products of NTA were also examined. The methyl esters of methyliminodiacetic acid (MIDA) and iminodiacetic acid (IDA) had much shorter retention times than the trimethyl ester of NTA. The possibility of adapting the present method to measure MIDA and IDA is being considered with a view to studying natural breakdown products of NTA.

The identification of the trimethyl ester of NTA was confirmed by using a mass spectrometer–gas chromatograph combination. Solutions of pure NTA in distilled water and samples of lake water, previously treated with NTA and exposed to natural conditions for some time, were esterified as described and analyzed.

Fast mass spectra scans were run, at both high and low sensitivities of the solvent peak, of the background and during the elution of the NTA peak. A large-scale sample of the NTA trimethyl ester prepared in the laboratory was also analyzed directly on the mass spectrometer probe (Fig. 3). Using the scans as a "fingerprint," the spectrum of NTA from lake water samples did not differ from that of the pure NTA trimethyl ester. Spurious peaks, which are not present on the probe sample, are found to be caused by background bleeding from the chromatographic column.

Tentative identification of all the major and minor peaks between m/e = 75 and 250 was in good agreement with that expected from a breakdown of the NTA molecule by electron bombardment. The complex picture at low m/e values was not elucidated fully, since these low molecular weight ions are almost always present in a mass spectrometer scan.

The relative ratios of the high m/e lines to the base line (m/e = 174) showed some variation that was correlated with the position on the peak where the scan was made. Scans at a higher sensitivity of the trailing shoulder of the NTA peak gave a parent peak at m/e = 247. This suggested the presence of a monoethyl dimethyl ester, which could be accounted for by traces of ethanol in the methanol HCl reagent. The absence of an additional pattern of lines, or "fingerprint," also indicated that there were no impurities in the NTA trimethyl ester peak.

Chromatography was found to be relatively straightforward and only minor adjustments to

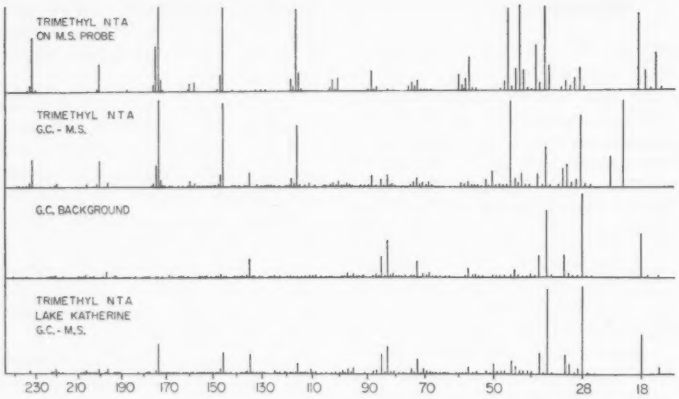

FIG. 3. Mass spectra scans of nitrilotriacetic acid (NTA).

the flow rates were required to produce identical chromatograms from two similar columns prepared from the same packing material. There was a minor problem in that the first injections of the day and those on new columns did not give the expected size of peak for internal standard and NTA. This was possibly due to adsorption phenomena and was overcome by injecting larger than normal amounts of NTA and internal standard before analyzing standards or samples. There was also some tailing of the solvent peak, but the column conditions were chosen so that the tailing was at an acceptable level by the time the internal standard and the NTA peaks were eluted, some 8–10 min after injection.

Regression analysis of duplicate determinations showed the correlation coefficient to be 0.998 over the whole range of concentrations. Regular calibration with known concentrations of NTA ensured that the ratios were reproducible and that the internal standard remained constant.

The storage efficiency and the recovery of NTA were tested by analyzing two different lake water samples before and after 6 weeks storage in a freezer and by the addition of increasing amounts of NTA (Fig. 4). The results showed that the method of storage was satisfactory and the recovery of NTA was within the limits of experimental error.

The method could detect about 25 µg/liter of NTA (Fig. 5). This was achieved by the initial concentration during freeze-drying, by running the gas chromatograph at the highest acceptable sensitivity for the NTA peak, and where necessary, allowing the samples to evaporate to about 50 µliters after the initial ratio between the internal

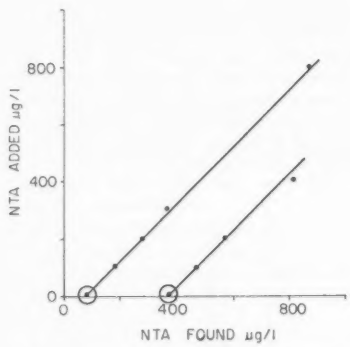

FIG. 4. . Recovery experiment of nitrilotriacetic acid (NTA). ○, NTA concentration on fresh sample; ●, NTA concentration after 6 weeks' storage and after addition of known amounts of NTA.

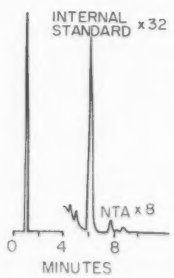

FIG. 5. Chromatogram of water sample containing 16 µg NTA per liter.

standard and the NTA ester was established. This last step also minimized the amount of solvent injected and the resultant tailing.

Acknowledgments

The authors acknowledge the generous help and advice given by Mr R. B. Stewart and Mr D. Bell of the Whiteshell Nuclear Research Establishment in the MS–GC analyses and in the interpretation of the data. We are also indebted to many colleagues at the Freshwater Institute for criticism, advice, and encouragement.

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NOTES

An Interstitial Water-Sampling Receptacle for Intertidal Mud Flats

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CREASER, E. P., JR. 1971. An interstitial water-sampling receptacle for intertidal mud flats. J. Fish. Res. Bd. Canada 28: 1049–1051.

In the apparatus described, a common receptacle may be used in conjunction with a pressure-compensating chamber or a vacuum service unit. The pressure-compensating unit is operable at any stage of the tide and requires from 12 to 24 hr to obtain a sample. The vacuum extract unit is operable on the exposed flat at low tide only and usually requires from 20 to 30 min to obtain a sample.

Received December 30, 1970

INTERSTITIAL water has been extracted from sediments by a variety of methods. These include draining (Bruce 1928), hydraulic or mechanical squeezing (Manheim 1966; Kruikov and Komarova 1954; Siever et al. 1961; Siever 1962), gas mechanical squeezing (Hartman 1965; Presley et al. 1967), and filtration through a porous material (Ishida 1953; Jones 1955; Borden 1931; Wiszniewiski 1934; Pennak 1940; Brafield 1964; Eriksen 1963; and Cole 1932).

The filtration principle used by Cole (1932) is of interest because it is similar to that of the present study. In essence, Cole's receptacle consisted of a filter cone glued to a glass cylinder. His device was designed for use in a freshwater pond. It would be impractical for use in intertidal areas with a considerable tidal range because of the support problems imposed and the presence of floating debris. The receptacle used in the present study not only eliminates these problems, but is cheap, easy to construct, and can be used to obtain a supply of interstitial water from a relatively undisturbed intertidal substratum.

The apparatus consists of the receptacle and either extraction unit. The receptacle is constructed of PVC 1120 I.D. 1.5-inch pipe (Fig. 1A). The RA 98 alundum filter cone (B), with a pore diameter of 20 μ, is cemented to one end of the pipe with PVC cement. A piece of polyethylene connecting tube (I.D. $\frac{1}{8}$-inch O.D. $\frac{1}{4}$ inch) (D), passing through a #6 rubber stopper (C) is the connecting link between the receptacle and the pressure-compensating chamber (F) or the vacuum unit (J).

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The receptacle–pressure-compensating chamber unit (Fig. 1) functions as follows. As the tide rises above tube D, the air volume within chamber F decreases and sea water flows into it by way of the S-shaped tube (E). Because this sea water is confined to chamber F, it does not contaminate the interstitial water entering receptacle A through filter cone B. As the tide recedes, the compressed air within chamber F returns to normal volume and the sea water is forced out.

Interstitial water samples were obtained from various depths by increasing the length of PVC pipe used for A. Receptacles of 6, 15, 24, and 33 cm, sampling at depths corresponding to total receptacle length (filter cone included) and PVC pipes used for chamber F construction, were of approximately the same length. Assuming a maximum high tide of 4.6 m, using any length unit, the air volume decrease within chamber F would amount to approximately two-thirds the volume at sea level, it would never completely fill, and no contaminating overflow from chamber F into the receptacle would occur. This pressure-compensating chamber unit is operable at any stage of the tide, and 12–24 hr are required to obtain 40–50 ml of clear interstitial water.

The receptacle–vacuum service unit[1] (Fig. 1J) was used as supplied. Attempts to use the soil water sampler supplied for the collection of interstitial mud water samples were unsuccessful, and consequently receptacle A was used.

[1]The vacuum service unit and dial gauge adapter (cat. no. 2005-A and 2001 respectively) to be used with a soil water sampler (cat. no. 1900) were supplied by Soilmoisture Equipment Company, 3005 de al Vina Street, Santa Barbara, California.

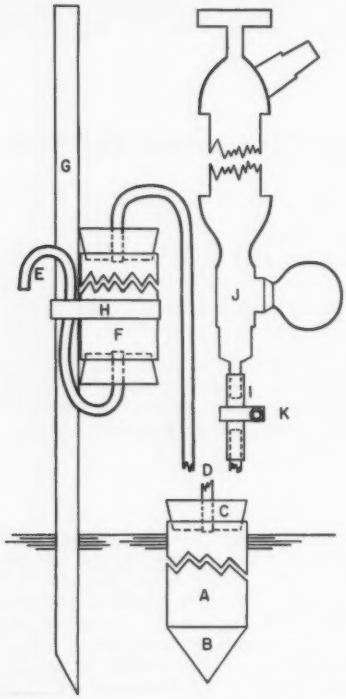

FIG. 1. The dual-functioning interstitial water sampling receptacle. A, receptacle; B, alundum filter cone; C, #6 rubber stopper; D, polyethylene connecting tube; E, polyethylene S-shaped tube; F, pressure-compensating chamber; G, wooden stake; H, rubber band; I, rubber tubing; J, vacuum gauge and service unit; K, screw clamp.

At the sampling site, receptacle A and vacuum unit J are connected by rubber tubing I. The receptacle is pushed into the mud, a vacuum of 70–80 cbars (52.45–59.94 cm of mercury) is applied to the receptacle by operation of the vacuum unit handle, and clamp K is closed. Unit J is then disconnected. A water sample is sucked into the receptacle, and 20–30 min later the remaining vacuum is released by opening clamp K. The receptacle is withdrawn, and the sample is poured into a plastic bottle for future analysis. The receptacle–vacuum unit combination is operable at low water only.

In the present study, the sampling device was used to obtain interstitial water for salinity analysis only. With minor modification, it probably could also be used for *p*H, *e*H, nitrate, phosphate, etc. The use of this receptacle–vacuum service combination for obtaining samples for O_2 analysis is in doubt.

Two precautions should be mentioned regarding use of either collecting method for obtaining salinity samples. First, the filter cones should be thoroughly cleaned in fresh water after each usage. A sonic vibrator and vacuum pressure pump proved useful for this. After the sediments in plugged pores were loosened with the sonic vibrator, tap water was both pulled and pushed through the filter cone by means of the vacuum pressure pump. Secondly, prior to reuse, the filter cones must be thoroughly dried, either in the air or in an oven set at a very low temperature. A hot drying temperature will cause the filter cones to crack at the base.

Failure to wash out the filter cones with tap water will result in salinity readings that are too high. If an unwashed cone is allowed to dry, salt remaining in the pores adds to the salinity of the next sample and results in a high salinity value.

Failure to dry the filter cones after being washed in tap water will result in salinity values that are too low. Tap water remaining in the pores, when mixed with the following sample, will tend to dilute the sample and result in a low salinity reading.

Even with the best of care the filter cones eventually become hopelessly plugged and must be replaced. Filter cone life can be increased by placing a piece of 9-cm no. 1 filter paper over the cones before pressing them into the mud.

The two collection devices were tested against one another to determine if they produced statistically similar results. Nine receptacle–pressure-compensating chamber units were set at 1-m intervals across a mud flat. The following day when these receptacles were removed, nine receptacle–vacuum units were placed in the same locality. Twenty minutes later these receptacles were also removed and all 18 samples were analyzed for salinity at the lab. The salinities of the nine samples collected by the former method were 29.63, 29.29, 29.47, 29.27, 29.33, 29.31, 29.09, 29.02, and 29.05‰; those collected by the latter method were 29.38, 29.22, 29.31, 29.31, 29.40, 29.31, 28.96, 29.02, and 29.16‰ respectively. A *t*-test using paired observations revealed that with 8 df at the 95% level of confidence, no significant difference existed between the results of the two methods ($t = .750$)

Acknowledgments — This study was conducted by the Department of Sea and Shore Fisheries in cooperation with the U.S. Department of Commerce, National Marine Fisheries Service, under Public Law 88-309, Project 3-16-R (Biological, Environmental, and Technological Research on Marine Worms).

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Maturity and Fecundity of the White Seaperch (*Phanerodon furcatus*) from Tomales Bay, California

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BANERJEE, T. 1971. Maturity and fecundity of the white seaperch (*Phanerodon furcatus*) from Tomales Bay, California. J. Fish. Res. Bd. Canada 28: 1051–1053.

The embryonic development of *Phanerodon furcatus* was found to be very similar to that of other members of the Embiotocidae; the smallest embryo (16.2 mm) had developed scales on the body and pigments in the eye. The embryos at birth measure approximately 43.5 mm. The average number of embryos for all females during the study was 19.6, and the embryos varied from 8 in a female measuring 196 mm to 33 in one measuring 262 mm in fork length. The number of embryos generally increases with an increase in size of the female parent. The breeding season of white seaperch, *Phanerodon furcatus*, in Tomales Bay, California, starts from the early part of May and extends to the middle of August.

Received January 12, 1971

THE white seaperch, *Phanerodon furcatus*, is a member of the family Embiotocidae. Regarding the early history of studies made on this family, Hubbs (1921) mentioned that "the viviparity of the embiotocids was first definitely made known by Dr. A. C. Jackson in 1853, in a letter to the elder Agassiz." Hubbs also has given an excellent account of a number of zoologists who studied this group in the 19th century.

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The white seaperch is distributed along the west coast of North America from Vancouver Island to Point Cabras, Baja California. This study was focused on the fecundity and embryonic development of *P. furcatus* collected from Tomales Bay, Marin County, California.

Methods — Embryos were collected by carefully splitting the abdomen of the female fish and removing the single-lobed ovary. The ovary was pressed from the anterior portion, allowing the embryos to fall into

a petri dish. The embryos were picked up by fine forceps and preserved in formalin. The lengths, weights, and dates of collection of the adult females were noted on a small tag and placed with the specimen. The embryos were counted and measured within 24 hr from the collection time. The 265 females sampled yielded 4977 embryos. A test similar to that used by Carlisle et al. (1960) was conducted for the length-frequency distribution of the embryos. The result showed that measuring only 10 embryos would represent the mean length of all the embryos (Fig. 1).

Results — In all embiotocids, the early development takes place within a cavity in the single-lobed ovary (Triplett 1960). Eigenmann (1894) reported that fertilization took place within the follicle. He also mentioned that the amount of yolk is greatly reduced, the cleavage approaches the holoblastic type, and the embryos are profoundly modified. None of the specimens I collected during this study showed any fertilized follicular eggs or noncleaved eggs in the ovarian cavity. In *P. furcatus* the embryos lie between 11 or 12 thin, highly vascular ovarian sheets. Embryos probably receive both nourishment and oxygen from these ovarian sheets, which are bathed by nutritive fluid (Carlisle et al. 1960). With the progress of embryonic development, the vertical fins of the embryos are greatly elevated. Similar observations have been made on other members of this family (Eigenmann 1894; Triplett 1960). The membrane of the elevated fin has been indicated by the above workers to be the main site for gaseous exchanges.

The collection of the embryos from the ovarian cavity and observations of the actual birth in the aquarium showed some emerging head first and others tail first. This head-to-tail orientation of the embryos allows them to conserve space within the ovarian sheets (Carlisle et al. 1960).

The size of the mother and the number of embryos present showed a linear relation (Fig. 2). Similar conditions were found by Carlisle et al. (1960) for *Amphistichus argenteus*. In parent fish ranging from 165 to 215 mm long, the average number of

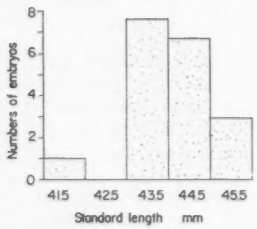

FIG. 1. Length frequency of 19 embryos from a 259-mm white seaperch.

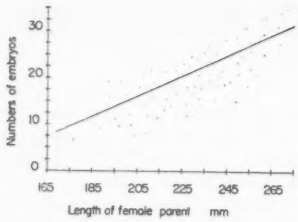

FIG. 2. Numbers of white seaperch embryos related to length of female parent, trend line fitted by eye.

embryos was 12.1, from 216 to 252 mm, 20.5, and above 252 mm 24.6. The average number of embryos for all females was 19.6. It varied from eight in a female measuring 196 mm to 33 in one measuring 262 mm. Smith (1964) reported observing 11 embryos in a *Phanerodon atripes* with total lengths of these embryos ranging from 34 to 36 mm.

I feel the above figures of the fecundity levels of *P. furcatus* are minimal, as the abortion rate in this species is very high. This was observed during collection, measurement, and transportation from the field to the laboratory, especially in those fish at their final term. A similar observation was made by Carlisle et al. (1960) for the barred surfperch. The high abortion rate may be due to trauma caused during collection.

Embryos of nearly every developmental stage were collected. Measurements of these embryos ranged from 16.2 to 44.5 mm standard length. Generally, the sexes of the embryos showed no difference in size. However, an occasional large or small embryo was observed. In the smallest embryo collected (16.2 mm) a clear pigmentation of the eyes was noticed, and scales had formed along the lateral line, below the pectoral fin, and around the eyes. The stage at which these structures first develop is not known. The average length of the embryos at birth was 43.5 mm.

Newly born fish were first seen in Tomales Bay early in May, and births occurred until the middle of August. Fish caught and examined by local sport fishermen at the mouth of the bay during late August were spent, whereas those from the southern part of the bay were still bearing embryos. Right after birth, the young school together near the shores of the bay.

The data accumulated from March to August shows an increase in embryo length during that period (Fig. 3). Embryos that were born in an aquarium showed no growth during a 1-month period of observation, probably due to the lack of food and space.

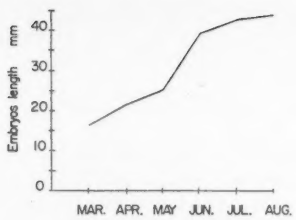

FIG. 3. Average monthly growth of white seaperch embryos.

Hubbs (1921) stated that in many genera of Embiotocidae the ratio of males to females appears to decrease with age, which holds true for *P. furcatus*. The percentage of males and females caught from January to July 1965 in Tomales Bay revealed a relatively high number of males in the months of January, February, and May–July, whereas in March and April they were almost absent in the catch (Table 1). This fluctuation in percentage composition may be due to two factors. First, during March and April the females are in their spawning stage and the males may leave the bay for the ocean. Second, it may be that the gravid females at their final stage of spawning tend to school together to find a suitable place for the birth of young ones. The catch during this period was probably only from these schools of females.

TABLE 1. Percent of male and female white seaperch caught from January 13 to July 28, 1965.

	Male	Female
Jan.	26.31	73.69
Feb.	20.00	80.00
Mar.	0.00	100.00
Apr.	1.34	98.66
May	43.08	56.92
June	35.62	64.38
July	39.68	60.32

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Genic Polymorphism of Tetrazolium Oxidase in Bluefin Tuna, *Thunnus thynnus*, from the Western North Atlantic

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EDMUNDS, P. H., AND J. I. SAMMONS. 1971. Genic polymorphism of tetrazolium oxidase in bluefin tuna, *Thunnus thynnus*, from the western North Atlantic. J. Fish. Res. Bd. Canada 28: 1053–1055.

Blood and tissues from bluefin tuna (*Thunnus thynnus*) contain several electrophoretically distinct forms of the enzyme tetrazolium oxidase. Every fish exhibits one of three oxidase phenotypes: a single band at either of two positions on the gel or both bands with a third midway between them. The pattern comprising three bands occurs in both males and females. Frequency distributions of phenotypes conform to Hardy-Weinberg distributions and apparently are not related to fish size. We conclude from these facts that bluefin tetrazolium oxidase contains two protein subunits and that phenotypic differences among individuals reflect segregation of two codominant autosomal alleles. Frequency distributions of phenotypes in three areas of the western North Atlantic do not differ significantly.

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GENETICALLY controlled protein polymorphisms, which are potentially useful in differentiating fish subpopulations (races), heretofore have not been identified in bluefin tuna, *Thunnus thynnus*. Barrett and Williams (1967) were unable to confirm an earlier report by Smith (1966) of individual variation in electrophoretic patterns of bluefin eye lens proteins. Nor did Barrett and Tsuyuki (1967) find individual variation in electrophoretic patterns of bluefin serum transferrin. Keyvanfar (1962) failed to identify blood factor differences among individual bluefin, but Lee (1965) and Gutierrez (1967) succeeded. However, they offered no hypotheses for hereditary control of the differences.

In this paper we describe variations in the electrophoretic form of an enzyme (tetrazolium oxidase) from blood and other tissues of bluefin tuna, present evidence that the variations are genetically controlled, and summarize preliminary data on the incidence of the variations among bluefin from three areas in the western North Atlantic.

Materials and methods — We examined samples of blood, heart muscle, skeletal muscle, or liver from 381 bluefin tuna, including 269 (3 lots) landed at Atlantic City, New Jersey; 87 (10 lots) landed at Galilee, Rhode Island; and 25 (2 lots) landed at Fox Point, Nova Scotia. New Jersey fish were caught during sportfishing tournaments in late June of 1968, 1969, and 1970. Two-thirds of the Rhode Island fish were caught during similar competitions in September of 1968, 1969, and 1970; the rest were taken aboard a charter boat in late July 1970. Nova Scotia fish were caught in commercial mackerel traps at the head of St. Margaret's Bay in July 1968 and August 1969.

For electrophoretic analyses we used 6% (w/v) acrylamide gels and either of two buffer systems: (1) a continuous tris (hydroxymethyl) amino methane (Tris)/EDTA/boric acid system as described by Peacock et al. (1965); (2) a discontinuous Tris/HCl/glycine system as described by Edmunds (1971). Electrophoresis of 10-μliter samples of red blood cell (RBC) hemolysate or tissue homogenate was carried out for about 3.5 hr at 350 v. We initially used a dehydrogenase-staining procedure (Bender and Ohno 1968) but switched to the more economical procedure (Brewer 1967) using only phenazine methosulfate and the tetrazolium dye, MTT, in the staining mixture. Other details of sample treatment in the field, and of preparation and electrophoretic analyses of samples in the laboratory, were as previously described (Edmunds 1971).

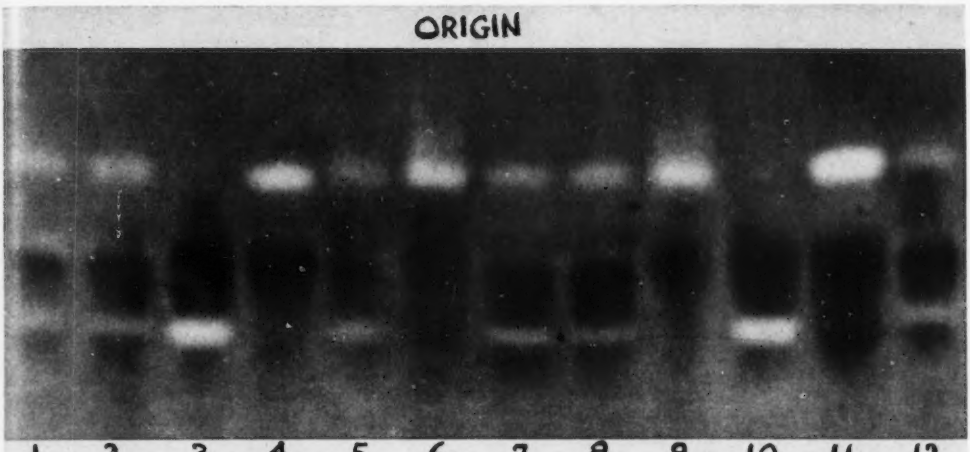
Results and discussion — During analyses of RBC hemolysates, we discovered three tetrazolium oxidase phenotypes (Fig. 1). Some fish have a single band at either of two positions on the gel and presumably are homozygous for one of two alleles, here designated Ox-1^A and Ox-1^B. All other fish have bands at both positions plus a third band midway between them and presumably are heterozygous. Existence of three bands instead of two in the presumed heterozygote suggests the Ox-1 enzyme is a dimer (Shaw 1965). The intermediate band is probably a hybrid molecule containing one unit each of Ox-1A and Ox-1B.

Our interpretation of observed phenotypic differences is based on several lines of indirect evidence. First, the variable Ox-1 system in bluefin resembles variable tetrazolium oxidase systems in humans (Brewer 1967), dogs (Baur and Schorr 1969), and Atlantic herring (G. J. Ridgway personal communication). Each system comprises three phenotypes, one of which contains elements of the two others. Genealogical evidence from humans and breeding experiments with dogs prove oxidase variants in these species are controlled by codominant alleles. Second, our population data do not differ significantly from the Hardy-Weinberg distribution, showing good agreement between theoretical frequencies of the three phenotypes based on this mode of inheritance and frequencies actually observed in different geographical areas (Table 1). Third, Ox-1 phenotypes apparently are unrelated to fish size; the observed distribution of phenotypes among 58 mature adult bluefin, which ranged in weight from 50 to 361 kg, is not significantly different from that for 29 juveniles, which ranged in weight from 3 to 23 kg (chi-square = 1.71, df = 2, $0.5 > P > 0.25$). Finally, occurrence of the three-banded, presumably heterozygous phenotype in both males and females eliminates any possibility of sex-linked inheritance.

Bluefin oxidase is fairly stable. Tissues from fish dead for less than 1 hr and tissues from fish dead for several hours produce equally intense patterns. Neither thawing and refreezing tissues twice before preparation of homogenates nor storage of homogenates for several months at –7 C have any detectable effect on Ox-1 patterns. The enzyme does not catalyze the Nadi reaction described by Brewer (1967) and therefore is not indophenol oxidase.

Oxidase patterns from homogenates of skeletal muscle, heart muscle, and liver (Fig. 2) are more complex than those of RBC. They exhibit Ox-1 variations but also have an achromatic band not detected in RBC hemolysates. It is slightly nearer the origin than Ox-1A and is more prominent in heart and liver than in muscle. Although it is more intense in some fish than in others, it occurred in all specimens that we examined, irrespective of Ox-1 phenotype. It is therefore not a part of the variable Ox-1 system.

Additional data from Rhode Island, Nova Scotia, and other widely separated areas such as



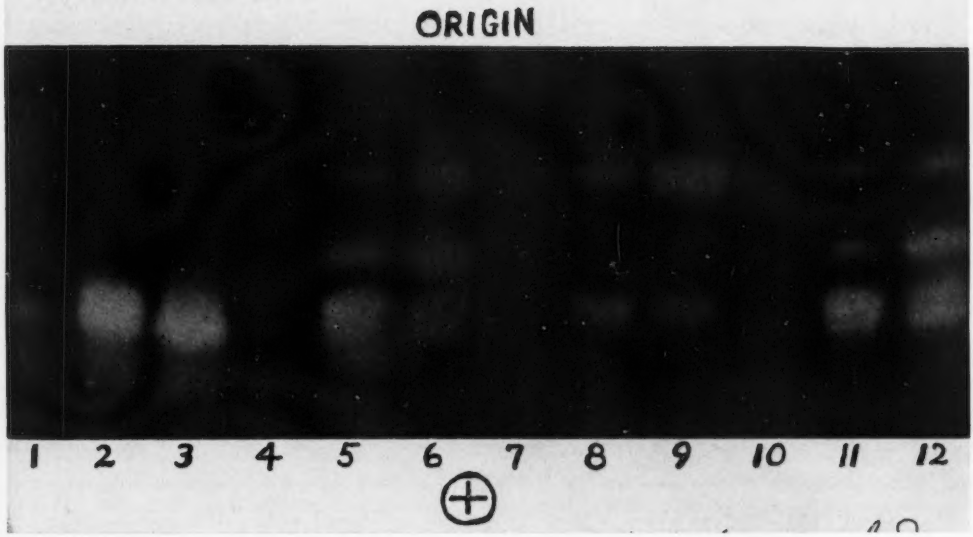


FIG. 1. (*Top*) Electrophoretic separation of red blood cell hemolysates showing (from left to right) Ox-1 phenotypes AB, AB, A, B, AB, B, AB, AB, B, A, B, AB. Hemoglobin partially obscures the intermediate oxidase band in phenotype AB.

FIG. 2. (*Bottom*) Electrophoretic separation of tissue homogenates from four fish showing (from left to right) muscle, heart, and liver with phenotypes Ox-1A(1–3), Ox-1AB(4–6), Ox-1B(7–9), and Ox-1AB(10–12). The oxidase band not detected in RBC hemolysates, which is not a part of the variable Ox-1 system, is prominent in positions 8 and 9, heart and liver, respectively, from a fish lacking band Ox-1A.

TABLE 1. Observed distributions of Ox-1 phenotypes in three areas and (in parentheses) theoretical distributions based on inheritance controlled by two codominant alleles.

Area	No. fish	Phenotype			P^a	X^2	Probability of worse fit df = 1
		Ox-1A	Ox-1AB	Ox-1B			
New Jersey	269	35	122	112	0.356	0.05	$0.90>P>0.75$
		(33.9)	(123.2)	(111.4)			
Rhode Island	87	13	37	37	0.362	0.55	$0.50>P>0.25$
		(11.4)	(40.2)	(35.4)			
Nova Scotia	25	2	13	10	0.340	0.64	$0.50>P>0.25$
		(2.9)	(11.2)	(10.9)			
Total	381	50	172	159	0.356	0.14	$0.75>P>0.50$
		(48.0)	(174.5)	(157.7)			

[a]Frequency of allele Ox-1^A estimated from observed distributions of phenotypes.

the Caribbean Sea, the eastern North Atlantic, and the South Atlantic may establish the value of the Ox-1 system as a racial characteristic. Data presented here do not. They do not provide evidence of more than one breeding population in the western North Atlantic. Perhaps only one exists there. On the other hand, our data do not prove that only one exists. Frequency distributions of Ox-1 phenotypes in the three sampling areas do not differ significantly (chi-square = 0.78, df = 4, $0.95 > P > 0.90$). But other genetically controlled systems may reveal subpopulations that are not apparent from comparisons based on the Ox-1 system.

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Determination of Fluoride in Soils by a Nonfusion Distillation *p*F Method

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KE, P. J., AND L. W. REGIER. 1971. Determination of fluoride in soils by a nonfusion distillation *p*F method. J. Fish. Res. Bd. Canada 28: 1055–1056.

The nonfusion distillation *p*F method has been successfully applied to the determination of fluoride in soils. The fluoride is distilled from the soil sample with $HClO_4$ and measured in the buffered distillate by an ion-specific fluoride electrode without any ashing or other separation operations. Soils or mud containing fluoride in the range between 1 to 700 ppm can be directly analyzed with a standard error of less than 1.5%.

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IN the analyses of rocks and soil for fluoride, the usual first step has been a separation of the silica. The method of Shell and Craig (1954) uses an alkaline fusion and removal of silica as a precipitate with zinc. The fluoride is then distilled and determined colorimetrically. Evans and Sergeant (1967) modified the method and eliminated the initial silica separation by using an anion exchange resin to remove interfering material after the distillation. The first of these methods suffers the disadvantage of loss or contamination during fusion (Hall 1968) and both of these methods are quite time-consuming.

Since a nonfusion distillation method had been useful for biological samples (Ke et al. 1969), when used in conjuction with the fluoride ion-

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specific electrode (Frant and Ross 1966), this same approach was applied to the determination of fluoride in soil and mud.

Apparatus and reagents — An Orion model 94-09 fluoride ion electrode was employed with an Orion 401 specific ion meter. The preparation of all reagents and the distillation apparatus were the same as reported previously (Ke et al. 1969).

Samples — Samples A and B were soil taken from two farms in Hants County, Nova Scotia. They were dried in a vacuum oven and ground to pass a 75-mesh screen. Samples C and D were core samples of sea mud obtained from a bay in Newfoundland that was receiving the waste effluent from a phosphate reduction plant. These samples were also vacuum-dried. All the samples were stored in polyethylene bottles.

Procedure — The method of Shell and Craig (1954) was used as basis for comparison.

The nonfusion distillation (NFD) *p*F method used was essentially the same as described previously (Ke et al. 1969). A 2–5-g sample of the soil was placed in the still with the mixture of $HClO_4$ and $AgClO_4$ solutions. The distillate was diluted with TISAB (total ionic strength adjusted buffer) (Ke and Regier 1971) and the fluoride activity measured with the ion-selective electrode.

Results and discussion — The results of a series of analyses by the two methods are compared in Table 1. The samples had varying amounts of added fluoride as internal standards or excess silicate as a potential source of interference. The fluoride content as determined by the NFD *p*F method was slightly higher than was found with the reference method. It is possible that some fluoride could have been lost in the fusion or precipitation steps in the reference procedure.

The addition of large relative amounts of silicate has no effect on the results of the analyses. In the reference method, this silicate was removed in the initial separation, whereas in the NFD *p*F method any silicate that was carried over in the distillation did not interfere. The electrode has been shown to respond to the fluorosilicate as well as the free fluoride ions (Ke et al. 1969).

The analyses of the four soil and mud samples are shown in Table 2. A high degree of precision was found in the analyses of these samples, even though the fluoride concentration ranged over two orders of magnitude. The standard deviation was about 1.5% of the concentration in sample B, which had only slightly over 1 ppm of fluoride.

Although the data reported here were obtained on samples that had been dried, drying is not necessary prior to analysis. The mud or soil sample can be weighted directly into the distillation flask with no prior treatment. This eliminates all question of possible loss of the fluoride prior to distillation.

The NFD *p*F method eliminates much of the time and a number of tedious manipulations necessary for the analysis of soil or mud samples for fluoride.

TABLE 1. Comparison of fluoride determination in sample A (5 g) with an internal fluoride standard or added silicate by the reference method and the NFD *p*F method.

F^- added (μg)	Na_2SiO_3 added (g)	F^- content, sample A (*ppm*) NFD *p*F method	Reference method
100	–	27.4	27.2
200	–	27.6	27.5
500	–	27.3	26.7
–	0.2	27.9	26.9
–	0.5	27.2	27.5
–	1.0	27.6	26.5
200	0.5	27.8	27.4
Avg		27.5±0.6 (SD)	27.1±1.7

TABLE 2. Determination of fluoride in soil samples by a nonfusion distillation *p*F method.

Sample	No. replicates analyzed	F^- content (*ppm* ± SD)
A	6	27.6±0.3
B	4	1.33±0.02
C	3	85.4±0.5
D	6	632.2±3.1

Acknowledgments — We thank Dr R. G. Ackman for the continued encouragement and Miss K. Rolfe for her assistance in these analyses.

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Survival of Young Fish in the Discharge Canal of a Nuclear Power Plant

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MARCY, B. C., JR. 1971. Survival of young fish in the discharge canal of a nuclear power plant. J. Fish. Res. Bd. Canada 28: 1057–1060.

No young fish of nine species entrained in the condenser cooling-water system of a nuclear power plant survived passage to the lower end of the plant's 1.83-km (1.14-mile) discharge canal when water temperatures were above 30 C. Temperatures in the canal remained above 30 C during 95% of the period during which the fish larvae and juveniles were collected near the plant's intake. A field observation device was developed to keep alive for counting those larvae and juvenile fish that survived passage to sampling points in the system.

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THIS report describes the effect of entrainment on nonscreenable young fishes in the condenser cooling-water system of the Connecticut Yankee Atomic Power Company's plant at Haddam Neck, Connecticut. This is the first reported study that attempts to determine the survival rates of fish larvae of less than 15 mm during passage through a condenser and discharge canal system.

Materials and methods — The plant is an electric generating station located 27 km (17 miles) from the mouth of the Connecticut River. The plant went into operation in October 1967. At peak load it pumps approximately 23 cm (825 cfs) of condenser cooling-water, which is heated to about 12.5 C above ambient water temperature. The plant uses approximately 6% of the average available river flow at the intake point during extreme low-flow conditions. A more detailed description has been provided by Merriman (1970).

The river water that enters the cooling-water system passes through (1) an intake structure at a velocity of 1–2 ft/sec, (2) a ⅜-inch mesh traveling screen, (3) twin, single-pass, divided water boxes — where the velocity may approach 8 ft/sec, (4) two 90° turns, and (5) two banks of 26,860 condenser tubes (0.9 inch I.D. × 600 inches long), in which the heat exchange takes place. Flow time is approximately 93 sec. The heated water then flows over a weir and continues down a canal 60 ft wide and 10 ft deep at an average velocity of 1–2 ft/sec depending upon tidal stage. The water reaches a discharge point in the main river 1.83 km (1.14 miles) south of the weir. The drop in temperature between the upper and lower ends of the canal is only 1 degree C. A nonscreenable organism is exposed to an elevated temperature in the canal for 50–100 min.

Gear used for the collection of fish specimens included a 0.5-m, No. 2 mesh (0.39-mm) cone-shaped nylon plankton net attached to a stainless steel ring with an impeller-type flowmeter at its center. A 1-liter plastic container was attached to the codend of the net. The apparatus used for maintaining water temperature and for counting living and dead specimens consisted of a portable, wooden water table (64.5 × 36.5 × 10.0 cm deep). A diaphragm water pump of 17 liters/min capacity drew water through a 2.5-cm diam hose to the table. Water depth in the table was maintained at 5.0 cm. Two 19-cm diam blackened preparation dishes were placed side by side in the water table to hold net-captured fish specimens for counting (at night by red light). A portable water table was essential in obtaining correct counts of living specimens, for small larvae died in minutes even with slight temperature changes. There was 93–96% agreement between specimen counts made in the field and those made later in the laboratory.

Net collections of larvae and early juvenile fishes were made from June 30 to July 29, 1970, at the plant's intake (A), below the outfall weir (B), and along the discharge canal at 457 m (C), 1220 m (D), and 1800 m (E) (Fig. 1). Triplicate 5-min samples were taken successively at the five stations. The data from each of the three collections per station were combined for comparisons. Three small collections minimized sampling error and held mortality due to net damage to a minimum. The intake station was sampled at its median depth and the discharge canal stations at the surface, middle, and bottom depths.

The percent survival at each discharge canal station was calculated by dividing the number of living larvae per cubic meter at each canal station by the number of living larvae per cubic meter at the intake for each sampling day. Survival was based on the number of living fish because sampling for dead fish in the canal gave inconsistent results in that they sank or floated because of their immobility or changes in specific gravity of the water. The sampling dates were selected so that they fell during the estimated peak abundance of the majority of larvae in the river. The daily injection of 13% sodium hypochlorite at the intake screen was

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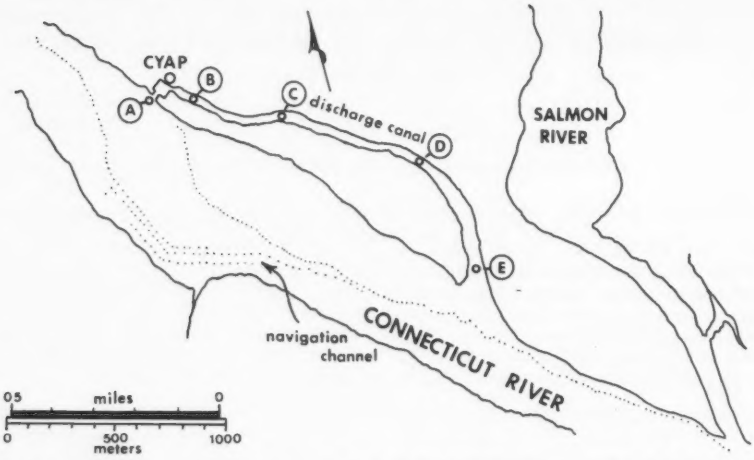

FIG. 1. Location of sampling stations A–E at the Connecticut Yankee Atomic Power Company (CYAP).

eliminated as a variable in the sampling by the appropriate selection of daily sampling times.

Entrained species were alewives, *Alosa pseudoharengus*, blueback herring, *Alosa aestivalis*, white perch, *Morone americana*, carp, *Cyprinus carpio*, white catfish, *Ictalurus catus*, American eel, *Anguilla rostrata*, spottail shiner, *Notropis hudsonius*, johnny darter, *Etheostoma nigrum*, and American shad, *Alosa sapidissima*.

Results — The survival rate of larvae on day 1, immediately after passing through the plant (station B), was 34.5%, and this percentage continued to drop to 7.5% at the end of the discharge canal. The survival rate on day 2 at station B dropped to 16.6% and no living specimens were collected at station D (Table 1). The majority of dead specimens were mangled, and this condition was more apparent in larger specimens. At temperatures above 30 C no living larvae or juveniles were taken at the end of the canal during the entire experiment. On day 3 and thereafter, with the canal temperature rising above 35.0 C, 100% mortality occurred during passage through the plant.

Data available from 1968 and 1969 plankton tows indicated that discharge-canal temperatures remained above 30 C during 95% of the season when the fish larvae and juveniles were abundant at the intake area:

Months sampled:	May	June	July	Aug.
No. fish/m³	.47	5.80	3.78	.08
%	4.6	57.3	37.3	.8
Mean discharge-canal temp (C)	22.3	30.3	35.1	35.0

Of the 2681 larvae and juveniles that were sorted, identified, and measured during the experiment, 97.5% were in the post-yolk sac larval stage (2.0–15.0 mm total length), and 2.5% were early juveniles (15.1–40.0 mm total length) (Table 2). Two small American eels (100.5–100.8 mm total length) were also taken. Alewives and blueback herring made up 97.6% of the total catch and white perch 1.3%. The six other species were represented in quantities of less than 1%. Most of the surviving fish that were noted on days 1 and 2 in the discharge canal were alewives and blueback herring. No living white perch larvae were observed after passage through the plant (station B). The only survivors taken in the canal after day 2 (canal temperature 37 C) were two American eels.

Discussion — This study showed that, at discharge-canal temperatures above 30 C, most fish larvae and juveniles that went through the plant were dead by the time they reached the end of the discharge canal. Mortalities that occurred in the canal were attributed to heat shock and prolonged exposure to elevated temperatures.

The only detailed studies on fish entrainment were reported by Kerr (1953) and Markowski (1962). Both studies showed no harmful effects on fish passage; however, both involved fish of larger sizes in discharge temperatures no greater than 28 C, and the total exposure time was less than 4 min. Coutant (MS 1970) presented the most comprehensive review of entrainment studies. Other important reviews of the subject were those of Naylor (1965) and Adams (1968).

TABLE 1. Percentages of larvae and early juveniles surviving at the various stations in the discharge canal, and environmental conditions, June 30–July 29, 1970.

Station[a]	Time (*hr*) and power level (*mwe*)	Temp (*C*)	Dissolved O_2 (*ppm*)	No. living/m³	No. dead/m³	% survival/m³
June 30						
A	9:00 AM–12:00 AM	22.2	8.1	1.74	0.91	65.5[b]
B	300	28.2	9.8	0.60	2.54	34.5
C		28.2	9.6	0.49	0.52	28.2
E		28.2	9.5	0.13	0.24	07.5
July 2						
A	12:00 AM–4:00 PM	22.0	9.2	0.54	0.30	64.8
B	588	33.5	9.0	0.09	1.64	16.6
C		33.5	9.1	0.10	1.22	18.5
D		33.0	9.2	0.00	0.96	00.0
E		33.0	9.5	0.00	0.82	00.0
July 6						
A	10:00 AM–3:00 PM	23.9	11.3	1.89	0.90	67.7
B	587	35.5	9.9	0.00	1.12	00.0
C		35.2	9.7	0.00	0.97	00.0
D		35.0	10.0	0.00	1.76	00.0
E		35.4	9.7	0.00	0.55	00.0
July 8						
A	2:30 PM–6:00 PM	29.0	11.5	0.92	0.14	86.8
B	584	38.5	9.2	0.00	0.96	00.0
C		38.0	9.2	0.00	0.78	00.0
D		38.0	9.4	0.00	0.92	00.0
E		37.8	9.8	0.00	0.26	00.0
July 8						
A	9:00 PM–1:30 AM	25.0	11.3	0.48	0.49	49.5
B	584	36.0	9.7	0.00	1.26	00.0
C		36.0	10.0	0.00	0.74	00.0
D		36.0	9.4	0.00	1.12	00.0
E		36.0	8.7	0.00	0.43	00.0
July 9						
A	2:30 AM–6:30 AM	25.5	10.3	1.41	0.86	62.1
B	581	37.0	9.3	0.03	2.19	02.6
C		37.0	9.5	0.00	0.97	00.0
D		36.5	9.6	0.00	2.06	00.0
E		36.5	9.4	0.00	1.19	00.0
July 22						
A	1:00 PM–4:00 PM	26.1	10.5	0.32	0.89	28.8
B	578	38.2	10.3	0.00	0.30	00.0
E		38.0	10.2	0.00	0.00	00.0
July 29						
A	9:00 AM–11:00 AM	30.0	7.7	0.04	0.00	100.0
B	569	41.0	9.5	0.00	0.33	00.0

[a]Station A is plant intake; B is outfall weir; C is 457 m below weir; D is 1200 m below weir; E is 1800 m below weir (end of canal).

[b]Intake (control) percent surviving netting and natural mortality factors. Percent survival at stations B through E was calculated by dividing the number of living larvae per cubic meter at each canal station by the number of living larvae per cubic meter at the intake (control) station.

Most fish collected in the present study were less than 15 mm long. It would seem impossible to effectively screen the small larvae without impairing plant operation. Kerr (1953) tried fine mesh screening and found an impingement of striped bass eggs and larvae for even a short time was fatal.

Acknowledgments — I thank the staff of the Connecticut River Ecological Study and the directors, D.

TABLE 2. Percentages of the total catches on the various sampling dates that were represented by the various species, and total lengths of the fish, June 30–July 29, 1970.

Date	No. collected	% of total catch	Mean length (*mm*)	Range
		A. aestivalis and A. pseudoharengus		
June 30	239	91.6	7.8	4.7–14.0
July 2	134	92.4	6.5	4.0–11.5
6	833	99.4	5.9	3.6–11.3
8	1277	97.9	9.3	4.0–40.0
22	115	100.0	10.1	5.5–28.5
29	18	100.0	8.4	5.0–13.5
		M. americana		
June 30	21	8.0	3.1	2.0–03.9
July 2	9	6.2	3.5	3.2–03.7
6	5	0.6	3.0	2.8–03.3
		C. carpio		
June 30	1	0.4	7.5	7.5
July 2	1	0.7	5.5	5.5
8	10	0.7	3.0	2.1–05.0
		I. catus		
July 2	1	0.7	10.6	10.6
8	2	0.1	17.2	13.0–21.3
		A. rostrata		
July 8	2	0.1	100.7	100.5–100.8
		N. hudsonius		
July 8	12	0.9	24.5	19.2–30.0
		E. nigrum		
July 8	1	0.1	28.5	28.5
		A. sapidissima		
July 8	1	0.1	16.0	16.0

Merriman and L. M. Thorpe. Drs J. W. Atz, M. R. Carriker, D. M. Pratt, E. C. Raney, and R. R. Whitney gave advice and critically reviewed the manuscript. Special thanks are due P. M. Jacobson and W. H. Dudek; also W. A. Boyd and the Essex Marine Laboratory for providing space and many services. The present paper is a product of the Connecticut River Ecological Study, which was undertaken by the Connecticut Yankee Atomic Power Company in fulfilment of the terms of the construction permit granted by the State of Connecticut Water Resources Commission on October 21, 1964.

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A Practical Method for Drying Freshwater Fish

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LANTZ, A. W., AND D. G. IREDALE. 1971. A practical method for drying freshwater fish. J. Fish. Res. Bd. Canada 28: 1061–1062.

A method and equipment suitable for drying fish in remote areas are described. Fish cut transversely into thin steaks dried faster and more completely than whole fillets, dressed fish, or minced fish. Fish were dried satisfactorily in about 7 hr on screen trays in a dryer constructed of plywood and accommodating an oil-fired space heater. The method is satisfactory for large and small quantities of fish, and may be applied to species with a limited sales potential that are frequently caught incidentally with more valuable commercial species.

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A simple method and equipment are proposed for drying small or large quantities of fish. The limited requirements for capital together with the uncomplicated nature of the equipment make it a convenient and practical method for small freshwater fishery operations. By varying degrees of equipment elaboration, the method can be adapted to drying on a larger scale. Species such as burbot (*Lota lota*), sheepshead (*Aplodinotus grunniens*), and white sucker (*Catostomus commersoni*) have limited sales potential and the quantities available are frequently not of sufficient volume to warrant reduction by conventional meal manufacturing methods. The proposed method of drying would be one solution to the problem of utilizing such species, which are frequently caught incidentally during normal fishing. In the present investigation a simple dryer was designed and tested to determine its practicability and efficiency.

This work was undertaken because it was considered necessary to have a method simpler than those available from the literature and more applicable for the widely divergent conditions encountered in the freshwater fishery. Legendre (1953) suggested a specific temperature and humidity for drying salt cod. Young and Sidaway (1943), Hamm et al. (1944), and Stansby (1945) have indicated that precooking, or precooking, pressing, and grinding, improved drying efficiency.

Initial drying experiments with northern pike (*Esox lucius*) indicated that drying could be accelerated sufficiently to inhibit bacterial spoilage if steaks were used instead of whole fillets or whole dressed fish. Steaks retained an acceptable flavour and odour throughout the drying process, whereas the whole fillets and the dressed whole fish became sour. Grinding dressed whole fish and whole fillets prior to drying proved unsatisfactory. Drying was very slow and the ground fish became sour and adhered to the supporting screen trays.

When steaks from several species were dried in a laboratory drying cabinet for 7 hr with temperature controlled at 125 F and relative humidity at 30%, ⅛- and ¼-inch thick steaks dried appreciably faster than ½- or 1-inch steaks, the percentage reductions in weight being as follows:

Thickness (*inches*):	⅛	¼	½	1
Burbot	76	69	51	41
Carp	72	67	53	34
Northern pike	75	71	49	38
Sheepshead	75	64	49	38
White sucker	72	66	44	33

Differences between species in drying time required to achieve a final reduction in weight of approximately 75% were relatively insignificant; however, steaks from fatty species required various extensions of the drying time.

A pilot plant model of the dryer (Fig. 1) was constructed from ¾-inch plywood sheets. Suitably positioned brackets support wooden-framed screen trays spaced approximately 6 inches apart. A standard oil-fired space heater provides a capacity of 27,000 BTU/hr. Fresh air passing through the heating chamber is warmed to maintain a temperature of 125 F in the dryer. Moisture-laden air is expelled from the stack.

When sucker steaks were dried in the model dryer, the temperature increased from 90 to 125 F as drying progressed. The natural air velocity was approximately 20 ft/min. Results of a typical run

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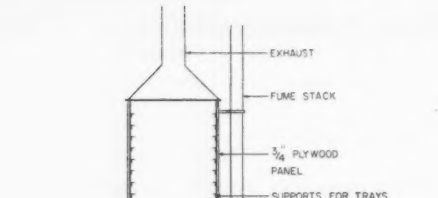

FIG. 1. A simple chamber for drying fish steaks.

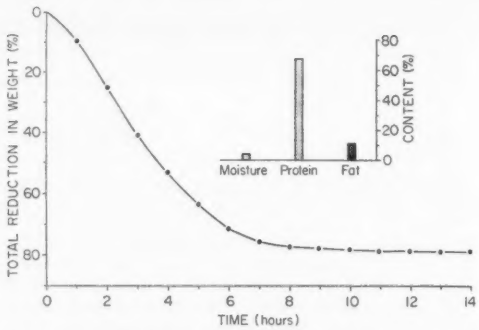

FIG. 2. Reduction in weight versus time, and proximate analysis of white sucker steaks dried in the model dryer.

(Fig. 2) indicate that a final weight reduction of 70–80% can be achieved in approximately 7 hr.

Fish dried using the technique and equipment when ground into meal had good colour and was free of objectionable odours. Nutritional qualities of the meals produced were not assessed. However, little damage to nutrients would be expected as a result of the mild drying conditions used.

To increase drying capacity while maintaining a reasonable degree of drying efficiency, a dryer could be built increasing the size in the lateral dimensions and retaining an overall height similar to that indicated in Fig. 1. A duplicated heat source or one with increased capacity would be required.

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A Sensor for Detecting the Presence of a Lobster (*Homarus americanus*) in a Burrow

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SCRIVENER, J. C., AND L. D. B. TERHUNE. 1971. A sensor for detecting the presence of a lobster (*Homarus americanus*) in a burrow. J. Fish. Res. Bd. Canada 28: 1062–1063.

The design and operation of a lobster sensor switch are described. It is a weight-sensitive device that forms the floor of a lobster's burrow. When connected to an event recorder, a continuous record of the presence of the lobster is obtained.

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THE American lobster (*Homarus americanus*) spends much time in burrows (Herrick 1909; Thomas 1968) from which it emerges to forage. During a laboratory study of lobster activity, an automatic monitoring system consisting of sensor and recorder was found useful and eliminated the need for continuous visual observations.

The sensor consists of two long, narrow, molded acrylic boxes, one pivoting inside the other. A normally open acrylic-encapsulated magnetic switch is attached to the bottom box, and a coated alnico magnet and counterweight are attached to the upper one (Fig. 1). When the upper box is depressed, the magnet closes the switch. This sensor forms the floor of a lobster's burrow and is triggered by an object placed in the middle of the platform. The sensor is connected to an event recorder so that a continuous record of a lobster's presence or absence is obtained.

To eliminate the smooth surface of the acrylic and give the lobsters good footing, the platform was painted with black acrylic paint and sprinkled with sand.

By adjusting the counterweight, the sensor can be triggered by at least as small a weight as 25 g. This would be equivalent to a hard-shelled lobster 70 mm carapace length and weighing 250 g in air. Thus almost every adult wild lobster in the intermolt stage would trigger the sensor.

Weighted sensors operated continuously with little maintenance for 7 months in a 20 × 10 × 4-ft tank with a coarse gravel and rock substrate. To prevent jamming, sand and shell that slipped down between the platform and support were removed fortnightly. When lobsters were given a choice, they did not appear to avoid burrows containing a sensor.

Acknowledgments — Funds and facilities for construction and testing were provided by the Nanaimo Biological Station. The encouragement of Dr R. J. Ghelardi was greatly appreciated.

HERRICK, F. H. 1909. Natural history of the American lobster. Bull. U.S. Bur. Fish. 29: 149–408.

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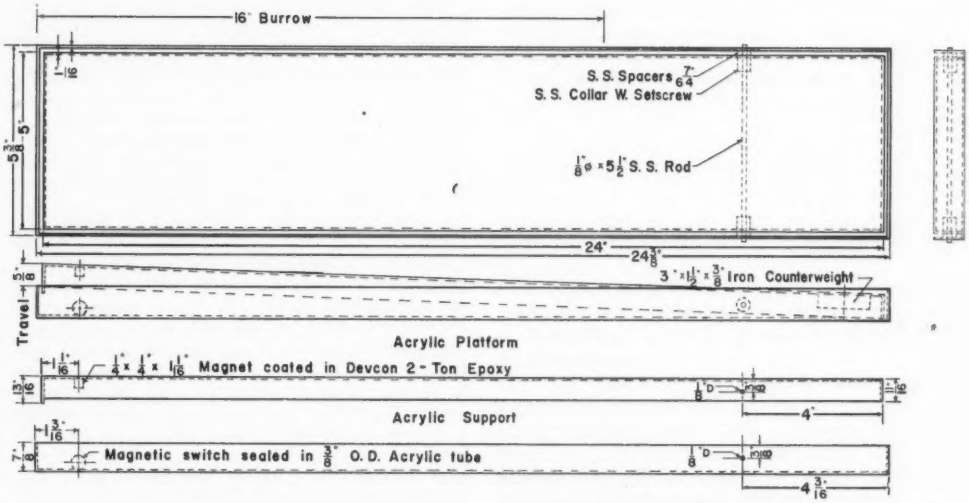

FIG. 1. Diagram of a lobster magnetic sensor switch. A G.E. 113 Magnetic switch was the most reliable of three switches tested. Grease-sealed P.V.C. insulated stranded hookup wire connected it to the 6-v recorder.

Infectious Pancreatic Necrosis Virus: Comparative Frequencies of Isolation from Feces and Organs of Brook Trout (*Salvelinus fontinalis*)

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FRANTSI, C., AND M. SAVAN. 1971. Infectious pancreatic necrosis virus: comparative frequencies of isolation from feces and organs of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Bd. Canada 26: 1064–1065.

When isolation of infectious pancreatic necrosis (IPN) virus was attempted simultaneously from feces and organs of brook trout (*Salvelinus fontinalis*), the virus was isolated more frequently from organs than from feces. However, the greater the degree of stress on the fish, the closer the number of isolations from feces appeared to approach that from organs, and the virus was isolated from both organs and feces of each of 10 fish accidentally exposed to low oxygen concentration for 4 days. The findings suggest that masked viral and bacterial infections may be detectable if fish are placed under stress.

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THIS study compares the value of feces and organs for the isolation of infectious pancreatic necrosis (IPN) virus from brook trout (*Salvelinus fontinalis*) and speculates on the value of placing fish under stress to detect masked viral or bacterial infections, or both.

IPN virus can be isolated from organs, feces, peritoneal washings, and seminal and ovarian fluids of infected fish (Wolf 1966). A higher frequency of isolation has been reported from feces than from peritoneal washes of brook trout (Billi and Wolf 1969).

During field investigations of the prevalence of IPN virus in brook trout from hatcheries and from stocked lakes in Ontario, the virus appeared to be isolated more frequently from homogenates of kidney, spleen, pancreas–pyloric cecae, and liver than from fecal samples (C. Frantsi unpublished data). In subsequent field work, isolation of the virus was attempted simultaneously from feces and organs of brook trout from the two sources. This was done only with freshly caught specimens, since autolysis may allow a spread of virus into the intestinal lumen giving erroneous results.

Both feces and organs were removed from fish and prepared as a 20% suspension with Hanks' balanced salt solution (HBSS) (*p*H 7.8–8.0). Feces mixtures from single fish were shaken and organs were ground with TenBroeck grinders, the suspensions were centrifuged at 650 *g* for 15 min, and a minimum of 0.2 ml of the supernatant was passed through 13-mm diam, 0.22-μ Millipore filters in Swinnex filter holders (Millipore Corp. Ltd.) onto HBSS-washed monolayers of RTG-2 cells. After adsorption for 30 min at 20 C, the fluid was removed, the monolayer washed with HBSS (*p*H 7.8), and the 1.0-ml maintenance medium added (Eagle's minimal essential medium with Earle's salts plus 10% fetal calf serum, 100 μg streptomycin, 100 units penicillin, *p*H 7.6). These cultures were incubated at 20 C for up to 1 week, and the medium was then passed through 0.22-μ filters onto a second monolayer of RTG-2 cells. Positive samples were confirmed by in vitro virus neutralization using rabbit anti-IPN serum. The 0.22-μ filters were used to avoid microbial contamination in cell cultures, since it was found that many microbes from soil and water environments would pass through 0.45-μ filters. The *p*H in cell cultures was maintained at 7.6–7.8 (suitable for IPN isolation) and the second culture passage carried out during all field investigations as necessary steps in screening for hemorrhagic septicemia (VHS) virus (Håstein et al. 1968), as yet unreported in North America.

The age of test brook trout ranged from 8 months (fingerlings) to 3 years. The lakes from which fish were taken had been stocked from 3 months (in the case of yearlings) to 1½ years previously (in the case of 2-year-olds).

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TABLE 1. IPN virus isolations from feces and organs of brook trout from hatcheries and stocked lakes.

Dates	Age (*months*)	No. tested	Isolations	
			Feces	Organs
		Hatcheries		
May 1969	15	25	4(16)[a]	10(40)
	16	50	20(40)	36(72)
Sept. 1969	36	79	1(1.2)	2(2.5)
May 1970	15	100	18(18)	24(24)
Oct. 1970	8	250/100[b]	32(12.8)	18(18)
Total or avg		504/354	75(15)	90(23)
		Lakes		
May 1969	26	5	2(40)	3(60)
June 1969	14	5	–(0)	2(40)
June 1969	14	3	3(100)	3(100)
Aug. 1969	16	6	3(50)	3(50)
Aug. 1969	29	1	0(0)	1(100)
Total or avg		20	8(40)[c]	12(60)[c]

[a]Values in parentheses are percentages.
[b]Only 100 fish were tested for organs.
[c]Because of the small numbers of fish collected in a manner suitable for this test, the percentage of shedders was incidentally high. The actual percentage was closer to 25 (determined by organ isolation and calculated from over 100 fish).

The virus was isolated more frequently from organs than from feces of the fish (Table 1). In all the fish for which the virus was isolated from feces it was isolated also from organs. In addition, although not indicated in the table, it appeared that the greater the stress the fish were under (for example, with an increase in water temperature or crowding (or both) in a hatchery as summer progressed) the greater the number of IPN shedders found and the closer the number of isolations from feces approached that from organs. The effect of stress was observed when 10 yearling brook trout were accidentally exposed to very low oxygen concentrations for a period of 4 days. These were from a population from which virus was isolated from the feces in 25% of the trout. Following the period of low oxygen, IPN virus was isolated from both feces and organs of all 10 of these moribund fish. This is further evidence that in a population with IPN shedders all fish are potential sources of the virus, Billi and Wolf (1969) having demonstrated by repeated sampling that over 90% of a test population of brook trout shed IPN at one time or another. We might also speculate that placing a few brood stock under controlled stress before sampling for viral or bacterial diseases, or both, may be an alternative to repeated sampling or to the large and costly sample size presently required (Amend and Wedemeyer 1970) for certification of a population of fish.

Acknowledgments — We acknowledge the financial support and excellent cooperation of the Ontario Department of Lands and Forests and their hatchery and field personnel.

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Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The number following each title refers to the FRB establishment listed below.

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(7) Fisheries Research Board of Canada
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Bedford Institute
P.O. Box 1006
Dartmouth, Nova Scotia

(8) Fisheries Research Board of Canada
Halifax Laboratory
P.O. Box 429
Halifax, Nova Scotia

(9) Fisheries Research Board of Canada
Biological Station
St. John's, Newfoundland

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